International Review of Tropical Medicine

EDITORIAL BOARD

INTERNATIONAL REVIEW OF

TROPICAL MEDICINE

Edited by

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Preface

The *International Review of Tropical Medicine* has been conceived in a period of rapidly changing economic social and technologic conditions throughout the world Advancement of man s economic social and technologic status has been accompanied by significant development indeed revolution in the management of his ills The art practice and science of medicine in the tropics which once were regarded as distinctive are now thought of in broader terms for research has shown diseases once considered as tropical to be prevalent also in temperate zones

Changing patterns and concepts of disease influenced by rapid events in other areas of man s activities emphasize that more and more the problems of health are *international* In a time shrunken world where a man may have breakfast in New York lunch in Paris and dinner in Cairo no longer can he afford to have one area of the world healthy and an adjacent one sick

The *International Review of Tropical Medicine* is dedicated to this international concept of world medicine and focuses its attention on tropical areas of the earth because here perhaps are the frontiers of the practice and science of medicine Here tribes are coalescing into nations peoples are emerging with new lives and hopes into a world where modern devices and knowledge can bring freedom from diseases Nowhere else in the world but in the tropics are there such vast and fertile areas of good earth rendered useless presently by disease organisms and vectors

The *International Review of Tropical Medicine* will provide critical reviews of knowledge of diseases that affect men or animals in tropical climates Diseases will be interpreted in their widest connotation to include those of an infectious metabolic or nutritional nature

I have just returned from a summer spent in several Central American countries where helminthic diseases outside of malnutrition (protein deficiency) are the area s most pressing health problem Broad spectrum efficient anthelmintics will be a major weapon in helminthic war but even the best of chemotherapeutic agents *is naught in the absence of sanitary* water supplies sanitary disposal of night soil and the Saturday night bath!

This first volume offers several viewpoints R G Cochrane in his contribution appraising the present position of leprosy has made a strong plea for the establishment of a world center for the study of what he suggests might be better called a powerhouse for the stimulation of research teaching and training in leprosy throughout the world a registry of histopathology and a reference laboratory where the variegated patterns of leprosy can be appreciated This is a noble idea L C Faust has presented

several facets of amebiasis which within the last generation has come to be recognized as an ubiquitous rather than a tropical disease It thus illustrates the international character of our editorial policy

Sir Philip Manson Bahr has written of history and there will be many young and old who will gain inspiration from an examination of the romance surrounding the now almost legendary personalities of our early days

Harry Hoogstraal has ably written a strong case in support of international efforts for the study of ticks and tick borne diseases H A P C Oomen has added still another illustration of the multiple facets of our objectives with his exhaustive analysis of Xerophthalmia

The paper of Morris Goldman illustrates a further principle that we *propose to follow We hope to bring to our readers authoritative reviews* of subjects that represent frontiers in research Immunochemical diagnosis by fluorescence offers intriguing possibilities for assistance in the unraveling of many mysteries in field and clinic

Finally in this first volume we offer a word on sources of information on tropical medicine by Charles Wilcocks To many old heads this may appear elementary but to young physicians and aspiring young medical researchers in big and small centers of learning such a summary of principal source materials will be of value I suggest some of us who aren t so young might also find value here

We therefore have tried to offer an heterogeneous series of papers that will illustrate the chief objectives in bringing to you authoritative critical reviews of the state of medical practice and science internationally with primary emphasis on that vast new frontier of today—the tropics

You will find a list of papers you may anticipate for the second volume of the *International Review of Tropical Medicine* on a nearby page

DAVID RICHARD LINCICOME

Washington D C
October 1960

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A Critical Appraisal of the Present Position of Leprosy

R G COCHRANE

American Leprosy Missions Inc New York and Ministry of Health London England

I Introduction

As the subject of the first review of this new series leprosy is accorded the standing it deserves and is recognized as an integral part of medicine

For too long has leprosy been looked upon as a baffling problem mostly fitted for the attention of the philanthropist idealist Christian missionary and social worker and not one that has a rightful place in the halls of scientific medicine Even today when much more attention is being given to the disease there is still a strong tendency to delegate to this subject a secondary place in the study and teaching of medicine

Sir George McRobert lately Professor of Medicine at the Medical College University of Madras emphasized in his lectures that tropical medicine simply meant medicine studied and practiced under special conditions and in a particular environment peculiar to the tropical and subtropical parts of the world

It may be well at the outset of this article once again to underline the reasons why leprosy has for so long been the Cinderella of medicine One

of these is that its roots go deep into the past and the whole subject has become encrusted by tradition this has resulted in it being placed in an atmosphere of legend and superstition which has put dread into the heart and created a reluctance to delve into the subject Further—and I say this quite deliberately—leprosy has suffered from an association with religious ideas that were erroneous This has caused the whole subject to be equated with certain theological concepts with reference to sin in an age when sin itself has largely been explained away !

The writer feels that a word of explanation is necessary at this point Although in the early part of the century this erroneous Biblical interpretation of leprosy brought relief to a group of sufferers who sorely needed help today this religious approach may be a handicap to the scientific advancement of the study of the disease and a hindrance to better understanding of it It is a difficult task to dissociate the mind of the physician from the influence of past tradition and if this is so with regard to the medical profession it is a great deal more so with regard to the general public Nevertheless there are signs that leprosy at long last is being rescued from the dark dungeons of superstition and is being brought out into the clear light of day for it has now become a disease that is most amenable to treatment as a result there is an increasing interest being taken in it by all branches of the medical profession and a more intelligent understanding of the subject by the general public

But to create interest is one thing to direct the new enthusiasm into profitable channels is quite another In order to seek a parallel to the present situation in leprosy we should turn to that allied mycobacterial disease—tuberculosis When fresh enthusiasm was aroused through the discovery of effective antibiotic remedies in tuberculosis and particularly as a result of new surgical techniques in the exploration of the chest wall tuberculosis passed from the realm of superstition and dread into that of scientific medicine Over the past three or four centuries or more tuberculosis mental ill health and leprosy have been considered to be diseases quite apart from ordinary ones and have cast terror into the hearts of men resulting in the ostracism of the sufferer Tuberculosis was the first disease to be rescued from superstition but this was possible only through the fresh enthusiasm that was aroused by the promise of effective treatment The surgical approach to tuberculosis through the advances in surgical techniques opened the eyes of the general public to the fact that tuberculosis need not always defeat man and that man had now the means by which he could effectively combat this scourge—a scourge that until fifteen or twenty years ago was of mortal significance

Mental health also is receiving attention through the newer under

standing of mental processes and the action and counteraction of bio chemical factors and of tranquilizing and other drugs As a result a fresh impetus has been given to the study of mental ill health

It should be pointed out that the three great social diseases of the past—tuberculosis mental illness and leprosy—through the patient work of devoted scientists are now better understood Because of this words such as consumptive and lunatic have definitely been put out of court because they convey a sense of social stigma disgrace and shame In the case of leprosy at every International Congress of Leprosy since 1931 resolutions banning the word leper in scientific usage have been pre sented It was at the International Congress of Leprosy at Madrid in 1953 that a resolution was finally adopted There is no doubt that this word signifies a conception of the disease that not only is detrimental to scientific progress but perpetuates an idea that encourages fear and a wrong approach on the part of the general public and even on that of the medical profession It should however be pointed out that the writer of this review is in agreement with the majority of leprologists in not accepting the term Hansen s disease as a synonym for leprosy The term is inaccurate for although Hansen discovered the bacillus he was not the first to describe the disease Furthermore the practice of naming diseases after those who first describe them is falling into disuse I personally would like to introduce the term mycobacterial reticulosis as a more scientific name for leprosy Although I am aware that the mycobacteria in general are invaders of the reticuloendothelial system nevertheless *Mycobacterium leprae* is probably the best illustration of the commensal existence which mycobacteria establish in the reticuloendothelial system Leprosy appears to attack the system in a peculiar and particular manner and lepromatous leprosy is truly a parasitization of the whole of the reticuloendothelial system Further it appears that leprosy would be a comparatively benign disease if it were not for the reactions of the tissues against this parasitic invader in its attempts to establish itself in the reticuloendothelial system Therefore I submit that a term such as mycobacterial reticulosis is a more accurate name for the disea e than leprosy The former conveys to the mind what the disease actually is whereas the word leprosy is etymologically incorrect This word literally means scaly or peeling and would more accurately apply to such diseases as psoriasis

II Etiology

It is now generally accepted that the cause of leprosy is a bacillus—*Mycobacterium leprae* This is not the place to describe in detail the bac teriology of this organism or to discuss its taxonomic status in the bacterio

logical world but it might be useful to summarize the history of *Myco bacterium leprae* and to indicate certain interesting developments in the field of artificial culture and animal inoculation

Mycobacterium leprae was discovered by Hansen in 1872 and the re sults of this work were published in 1874 twelve years before the dis covery of *Mycobacterium tuberculosis* by Robert Koch in 1886 It was the work of Hansen that stimulated Koch to search for a similar organism in tuberculosis *M leprae* is of particular interest because it has remained a challenge to the bacteriologist Up to now it has not been definitely shown to have been cultivated on artificial media neither have any animals been infected with the organism Many claims however have been made by numerous workers that *M leprae* has been successfully cultivated Among these are those of Freire (1956) who maintained that he produced a growth of *M leprae* on a slide culture using a neutral medium consisting of Kirchner s solution with 0 1% agar and 20% of lepromatous plasma or serum Eleanor Alexander Jackson (1953) has continued to work along these lines and the claims are at present being investigated Sister Marie Suzanne (1953) caused considerable interest in the scientific world by the publication of her work in connection with the therapy of leprosy but I think that it can be generally concluded that the mycobac terium that she has grown is not *M leprae* although it may be closely allied to it There is some evidence that therapeutically it may act as an antigen adjuvant and therefore this may explain the occasional success reported as the result of administering her vaccine

At the International Leprosy Congress (1958) held at Tokyo very interesting developments were reported by Chatterjee (1958) of Calcutta in connection with certain animal inoculation experiments Dr Chatterjee used a special cross bred black mouse for this work he claimed that he was able to produce a growth of *M leprae* in these mice and that this organism was transmitted to other animals He concluded that his experiment seemed to be successful The evidence that Dr Chatterjee has put forward is of great interest and I have had the pleasure of seeing this work in Calcutta It appears to me that there has been a definite multiplication of *M leprae* within the tissues of these mice and that a laboratory animal has been found in which it is possible to grow *M leprae* and to maintain growth for a considerable period of time

In this connection one must also mention the work of Gunders (1958) in Liberia who has claimed to have infected a chimpanzee with leprosy It appears to me that Chatterjee (1958) and the other workers have pro duced a multiplication of *M leprae* in animals but there is at present—except perhaps for the work of Gunders—little evidence that progressive

disease has developed in any of the animals Previous work on animals particularly that of Cochrane *et al* (1945) in Madras has shown that under limited circumstances an animal will occasionally show multiplication of *M leprae* and even parasitization of the reticuloendothelial system but it also showed that if the animal were kept alive for a sufficiently long period the multiplication of the organism gradually slowed down until it ceased altogether and finally the bacilli disappeared from the reticuloendothelial system of the animal Furthermore I think it can be said that it would be unlikely for an animal to show signs and symptoms similar to those seen in human leprosy but if an animal were successfully inoculated the disease produced would be much more likely to be of the nature of that seen in rat leprosy In other words *M leprae* may multiply in the reticuloendothelial system but does not show the characteristic clinical manifestations seen in human leprosy

It will be interesting to follow up the work of Chatterjee (1958) to see whether in the hybrid mouse *M leprae* continues to multiply in the reticuloendothelial system of the animal over the whole span of the animal s life Dr Chatterjee s experience may be similar to that of others in that multiplication commences in the reticuloendothelial system which becomes invaded and the animal appears to be a suitable one for the study of infection but at the end of varying periods [in the monkeys of Cochrane *et al* (1945) it was six months] the bacilli cease to multiply and ultimately disappear from the tissues I personally would not be surprised if this happened in the tissues of these hybrid mice of Dr Chatterjee Nevertheless if a growth of *M leprae* can be maintained in an animal for a suitable length of time this would be significant and would be a great help in the testing of drugs for experimental screening purposes It has been shown by Rees and Wong (1958) that it is possible to secure limited multiplication of *Mycobacterium muris murium* under certain conditions and Robson and Smith (1959) have produced a limited growth of this organism on the cornea of mice Attention should be drawn to recent work done by Binford (1959) with reference to the inoculation experiments in Syrian hamsters Following up the generally noted observation that *M leprae* prefers anatomic sites of relatively lower temperature rather than the internal organs he chose the external ear tail foot testis scrotum and skin as inoculation sites Binford showed that histiocytic granulomatous lesions resembling human lepromatous leprosy in histologic pattern were obtained in the testes and ears of the golden hamsters approximately eighteen months after inoculation and that preliminary observations after five months indicated successful transfer of the infection to other hamsters It was of further interest to note that of other animals

chosen including guinea pigs white rats white mice and hairless mice in none did the inoculation experiments carried out on similar lines succeed This is of significance in view of Adler s work some years ago in which he claimed infection with *M leprae* by inoculating Syrian hamsters after splenectomy

While progress is undoubtedly being made in our attempts to establish an infection of *M leprae* within the tissues of an animal equally interest ing results have been reported on experiments on the growth of *M leprae* in artificial media The work of Khanolkar (1951) should be mentioned and it is of interest to note that Cochrane (1954) made the following statement some years ago The predilection of *M leprae* for nerves in the early initial phases points to the possibility that *M leprae* can only be come pathogenic in man after passing through the superficial nerve plexus of the skin This suggested passage through the nerve tissue opens up in triguing possibilities in relation to fresh approaches in the attempt to culti vate *M leprae* Khanolkar (1951) was able to demonstrate that *M leprae* appeared in very early stages of infection in the small cutaneous nerve plexuses of the skin His colleagues were able to cultivate an acid fast organism similar to *M leprae in vitro* using human fetal spinal ganglion cultures in the substrate An acid fast organism isolated from several cases of lepromatous leprosy and grown on stromal fibrocytes from human spinal ganglia or on a modified fluid of stock cell cultures several months old adapts itself slowly to bacteriological media usually employed for the growth of *M tuberculosis* Rees and Wong (1958) have confirmed that this mycobacterium is taxonomically different from any mycobacterium which has previously been described Further work will be needed to verify whether it is actually *M leprae*

All this work adds undoubtedly to our knowledge of *M leprae* and it is sincerely hoped that as a result the day has been brought nearer when *M leprae* will have been successfully grown on artificial media and an experimental animal suitable for laboratory experiments will have been discovered

III Epidemiology

Although it is universally accepted that *M leprae* is the causative organism of leprosy it is of interest to review the question of the factors necessary in the acquirement of clinical signs of the disease after the tissues of the human body have been challenged by *M leprae*

There is a growing amount of evidence indicating that the skin is the point of entry of *M leprae* and that from the skin the organism is prob ably rubbed into the finer nerve terminals and is first seen in the cells

of Schwann The Schwann sheath apparently extends to the prickle cell layer of the epidermis but in this situation it appears to wrap itself round these cells as a very fine almost imperceptible membrane Therefore it is not difficult to conjecture that it would be easy for the bacilli to penetrate through the epidermis into the Schwann sheath and then into the Schwann cells Furthermore the axis cylinders at this point are in close proximity to the Schwann sheath so that there is no visible separation and therefore the parasite could also find entrance into the axis cylinders of the nerves It is apparent from the work of Khanolkar (1951) and Weddell *et al* (1959) that blebs or saccules develop in connection with the Schwann sheath and the axis cylinders and that these contain *M leprae* which ultimately burst into the surrounding tissues The further development of the disease therefore depends on the interaction between the tissues and the bacillus These concepts will be discussed later

It follows therefore that if the skin is the most important organ in the requirement of leprosy it is important to consider what type of contact is necessary for infection and for the development of the disease the essential question is How long must this contact continue if an individual's resistance is to be sufficiently altered for the bacilli to produce a breakdown of the defences of the body and the resulting signs of clinical leprosy

All the evidence goes to show that it is the open case of leprosy which is infective and that the closed case is generally speaking noninfective By an open case of leprosy is meant that form of the disease in which *M leprae* can be isolated from the skin by routine methods of examination If this definition be accepted it follows that any case in which the bacillus can be demonstrated in the skin or the mucosa is either actually or potentially an open or infective case although it is true that lepromatous leprosy contains as a rule the largest numbers of organisms in the skin it does not follow that lepromatous leprosy is the only form of the disease in which *M leprae* can be passed to a healthy member of the community The tendency for certain forms of leprosy for example the dimorphous macular lesion and the so called Poussee bacillifere of the Belgian workers (a form of tuberculoid leprosy) to pass through positive phases largely explains the contention by Davison (1949) McDonald (1951) and others that nonlepromatous leprosy must be considered infective If therefore it is accepted that the skin is usually the site of entry of *M leprae* the next question arises How much contact is necessary for an individual to develop the disease? The development of a particular disease requires not only the introduction of the causative organism into

the tissues but there are many other factors that influence the acquirement of the disease

It has been maintained for a considerable time that intimate and prolonged contact is necessary before a person can be infected with leprosy It is however very difficult to say exactly what is meant by intimate and prolonged contact This may signify weeks months or years In this connection Badger (1959) states that it is recognized that an individual who has had intimate contact over a long period of time will be more likely to become infected than one who has had only casual contact with an infectious case There has been a considerable amount of discussion over the past years as to the amount of contact necessary and as to the intimacy of that contact but the main fact that has come out of these discussions is that leprosy is a contagious disease and is acquired when a case which is discharging bacilli from the skin or mucous membrane of the nose comes into contact with a healthy and susceptible member of the community

There is much accumulated evidence to show that contact is the main factor in the acquirement of leprosy and it is of interest to note that this is emphasized by the appearance of certain initial lesions in leprosy For instance in certain parts of Africa one of the sites for a primary infection is the center of the forehead whereas in other parts of Africa this is not seen nor is this initial lesion seen in India or in Korea If one investigates the methods by which children are carried in these countries it is noted that in Africa where the initial lesion is not infrequently seen on the forehead the mother carries the child on her back and as the back is naked the skin of the child s forehead is constantly being rubbed against the infective skin of the mother s back In Korea although the child is carried in exactly the same way the mother is clothed and the initial lesions appear on the child s arms or other parts more exposed to handling In India the more common sites for initial lesions are the thighs and buttocks for mothers carry their children on their hips in that country Further Rogers and Muir (1925) have brought forward evidence to show that a child after constantly lying on an infective pillow may develop a primary lesion on the cheek

Workers particularly in India have emphasized age as one of the most important epidemiological factors and as far as India is concerned this is true Cochrane (1947) made the statement that the majority of persons acquire leprosy in India before the age of 20 and [that] many have been infected by the time they reach fifteen years of age Recently Badger (1959) has disputed that age is an important factor in the epidemiology of the disease This contradiction apparently lies in the ob

servation that social customs differ sufficiently to explain this anomaly Cochrane (1959b) has pointed out that if these customs are studied it will be found that generally speaking in the East children tend to come into closer and more intimate contact with an infected parent or relative than do adults and in the West adults tend to come into more intimate contact with an infected person than children For instance in the East one seldom finds that husband and wife sleep in the same bed as a regular habit whereas a child will always sleep with one of its parents In the West however the general custom is that a husband sleeps with his wife in the same bed and the child sleeps in a separate bed This means therefore that a child in the East is much more intimately and continuously in close contact at night with an adult than one in the West and it must be admitted that figures analyzed by Badger (1959) show quite definitely that as far as America and Hawaii are concerned leprosy is not necessarily a disease of children It can be concluded therefore that the most important factors in the transmission of leprosy are the presence of open cases in sufficient numbers and the opportunity for contact with the sources of infection The greater the intimacy of contact the greater the risk of becoming infected

Other factors have been incriminated in the acquirement of leprosy These include family susceptibility race diet climate sex and indirect contacts by means of insect vectors In the opinion of the writer none of these is of paramount importance in the acquirement of leprosy compared to the importance of direct contact with an infected individual

Kinnear Brown (1959) has suggested that susceptibility to leprosy may be related to some complicated genetic factor Rotberg (1937) and others have surmised that the susceptibility of leprosy may be due to what he calls an N factor It may be that this type of susceptibility is inherent in the genes Therefore it would be difficult indeed to produce evidence that there is a family susceptibility or a genetic factor in leprosy without initiating a very complicated enquiry In an ordinary analysis of cases arising in any particular neighborhood family susceptibility is not a factor that strikes one unduly Since however the day when Jonathan Hutchinson (1906) incriminated fish eating as an epidemiological cause of importance in the acquirement of leprosy diet has assumed a place of importance in the epidemiology of the disease Although it is not disputed that in certain forms of leprosy particularly advanced lepromatous leprosy as seen in the Mongolian and Caucasian races a gross protein deficiency has to be taken into account—for it is a factor which is liable to result in a nutritional state that not only is detrimental to the healing of the disease but may produce secondary ulcerations which terminate in death

—in the opinion of the writer this is due rather to the disease causing an imbalance in the protein metabolism than to the absence of an adequate amount of protein causing this manifestation of the disease

Skinsnes (1958) in the recent International Congress in Tokyo emphasized the importance of diet in this respect Nevertheless as a straight epidemiological factor it is extremely difficult to incriminate diet as a basic factor in the causation of the disease Cochrane *et al* (1940) concluded after a four years study of dietetic factors in leprosy that no relationship could be discovered between diet and the progress of the disease or the acquirement of the more serious forms of leprosy and that once leprosy has been established in a community an improvement in diet does not show a corresponding improvement in the incidence of the disease or result in a marked difference in treatment

Cochrane (1935) and Lowe (1938) *have pointed out that generally* speaking the lighter colored races not only tend to have a higher incidence of lepromatous leprosy but that the results and progress under treatment are not as satisfactory as for the darker races This raises the *question of racial susceptibility It can be said that the incidence of* lepromatous leprosy is higher in the lighter colored races than in the darker but there is not sufficient evidence to indicate that one race shows a higher susceptibility to leprosy than another

When climate is considered it must be concluded that it plays little part in the acquirement of leprosy except that in areas of high humidity it is frequently noted that social customs are such that closer contact is seen between the healthy and the infected individual in a community

In summary then it can be said that leprosy is acquired in the main by contact with an open case of the disease—and this contact is usually more or less intimate such as sleeping in the same bed with a patient—or by any means by which the infected skin is rubbed against the skin of the healthy contact Indirect contacts such as bedding matting clothing etc must also be included in any discussion on epidemiology Therefore apart from the possibility of a racial genetic factor increasing the susceptibility of an individual there are probably no other factors of any importance in the acquirement of the disease and air food or water need not be considered seriously in any discussion on the epidemiology of leprosy

IV Immunology

The subject of immunology in our understanding of the defense which the tissues put up against *M leprae* has assumed an increasing importance in the general discussion of the development and spread of leprosy within

the body As in all diseases there are two forms of defense that one must discuss first natural immunity and secondly acquired immunity It is an observation of some importance that leprosy is one of the less contagious diseases which afflict mankind and that the susceptibility rate in a given community varies between 2 and 10% of the population This means that 90–98% of the human race is relatively nonsusceptible to the disease or have acquired a large degree of natural immunity Until now the subject of immunology has been viewed too much from the point of view of tissue hypersensitivity or potential hypersensitivity that is in terms of the lepromin reaction and not enough attention has been given to the subject of immunity in general In other words while the matter of tissue resistance or tissue immunity has been very fully discussed the question of natural immunity or nonsusceptibility has received scant attention until recently when as I have said fundamental research workers have taken an interest in the whole subject of leprosy Therefore in any discussion with regard to immunity from leprosy the two factors that must be brought under consideration are first natural immunity and second acquired immunity

A Natural Immunity

It is known that not all persons who are challenged with *M leprae* develop leprosy and in the human race the susceptible individuals appear to be between 2 and 10% of the total population This means that most human beings have a natural immunity to leprosy Either one of two things happens (1) *M leprae* is unable to penetrate the epidermis and therefore cannot be introduced into the fine nerve terminals or (2) when introduced the macrophages whether these be the wandering histiocytes in the corium of the skin or the Schwann cells are able to destroy the bacillus and thus the bacillus never gains a footing within the cell and cannot multiply

(1) It would be very difficult to prove that *M leprae* in certain instances cannot penetrate the skin and therefore cannot infect a human individual It is interesting to note however that two biopsies were performed on children who had been in contact with open cases [unpublished work] the children were of about the same age both had prolonged contact with the mother who was nursing them and who was with them all the time and both were sleeping with the mother at night In one child the biopsy revealed an early infection of the nerve endings of the skin suggestive of lepromatous leprosy whereas in the other child there was no such evidence This opens up an interesting possibility which can be confirmed only by the study of contacts of open cases of leprosy under all

circumstances and at all ages to see why in certain individuals even though there is contact with infection the organism cannot apparently penetrate the skin and establish itself

(2) The second reason for the nonsusceptibility of the individual may be that the macrophage cells of the reticuloendothelial system may not be a suitable medium in which *M leprae* can multiply and when they enter into these cells they are destroyed Hanks and Fernandez (1956) have shown *that a condition can be produced or perhaps stimulated in connection* with the allied organism of leprosy—*Mycobacterium muris murium*—*which results in the infection either being delayed or completely aborted* These workers reported that if mice were given inoculations of bacillus Calmette Guerin (BCG) together with a heat killed suspension of *M muris murium* then after these animals were challenged with live rat leprosy bacilli in a significant proportion of cases the disease did not develop and further if during the latent period of the disease the animals were inoculated with the same mixture the development of leprosy was delayed or aborted altogether He stated therefore that BCG vaccination combined with a heat killed suspension of *M muris murium* acted as an antigenic adjuvant *It is interesting to note that* Hanks (personal communication) has also suggested that hydnocarpus or chaulmoogra oil may *also act thus and this opens up an intriguing field for research which al*though difficult would give us important information as to the mechanism of natural immunity *On the other hand* Kinnear Brown (1939) may be correct when he suggests that natural immunity is a recessive genetic factor and he emphasizes this in a recent article which supports the ideas of Rotberg (1937) who referred to the N factor which some individuals appear to possess though in others it is lacking Fernandez (1943) has referred to this as protective allergy but had in mind that form of tissue defense in which the lepromin reaction is positive

B Acquired Immunity

When one is discussing the possibility of acquired immunity one has to admit that this as far as we know can be discussed only in terms of a *tissue response or a tissue defense* Although it is known that hypersensitivity does not necessarily mean immunity nevertheless the development of a *hypersensitivity or a potential hypersensitivity results in the body defending itself against M leprae* This defense is increasingly successful in proportion to the strength of the allergy *that the tissues can develop* It is altogether too large a subject to be discussed in detail in an article of this nature but the value of the lepromin test *in estimating the degree of* tissue defense has assumed importance within recent years It is suggested

that not only does a positive lepromin signify an adequate tissue defence but that possibly one can use repeated lepromin injections as a therapeutic remedy in the treatment of leprosy so that by treatment with injections of lepromin the response in the tissue will be changed and therefore the prognosis become correspondingly more favorable Ridley (1959) emphasizes the importance of taking note of the role of the lymphocyte in estimating prognosis and Kuper (1958) has suggested that by injections of lepromin the lymphocytic response is stimulated into activity and he therefore concluded that there may be a therapeutic value in repeated lepromin tests It is generally assumed that a positive lepromin reaction is of better prognostic value than a negative one and many workers particularly Chaussinand (1948) Fernandez (1939) and de Souza Campos (1953) laid stress on the importance of using BCG vaccination to transform a negative lepromin into a positive in persons who have contact with open cases of leprosy The subject is an extremely complicated one and the optimism with regard to the possibility of using BCG vaccination as a preventive inoculation has been somewhat dampened by the fact that it has been shown by Doull *et al* (1957) that lepromin testing will itself cause a transformation to a positive reaction Further these workers have shown that in the ordinary normal development of an individual from infancy to childhood there is a marked tendency for a lepromin positive reaction to develop in the tissues although they have not been challenged by *M leprae* As I have said elsewhere acceptance of the theory that positive lepromin induced by BCG vaccination or by any other means is effective in preventing the development of leprosy depends on whether one accepts the theory that the allergic response determines the type of the disease or considers that the type of disease determines the allergic response If the former statement is true it is advisable that everyone who shows a negative lepromin response should have this transformed into a positive but if the type of disease determines the allergic response transformation of a negative to a positive lepromin reaction may not be of any immunological significance One unfortunately has to leave this fascinating subject until more evidence is accumulated with regard to the question of natural and acquired immunity to leprosy

In any discussion concerning the subject of immunity in leprosy it should be noted that the mere circulation of antibodies is no indication of effective immunity The evidence supports the contention that the greater the amount of circulating antibodies the less able are the tissues to defend themselves against *M leprae* for it has been shown that in the active progressive forms of lepromatous leprosy in which the prognosis is poor there is a reversal of the serum globulin and the serum albumin

ratios in the body with an accompanying increase in plasma cells This indicates that an increase in circulating antibodies resulting in antibody formation is not a true manifestation of adequate immunity This alteration in the serum albumin and serum globulin ratios only signifies a bio chemical reaction which results in the stimulation of what might be termed chemical antibodies rather than any indication of the ability of the tissues to overcome *M leprae*

In concluding this discussion on the subject of immunity the question might be raised as to what happens in a lepromatous case that becomes negative for in such a case the ability of the tissues to develop an effective tissue response is absent because the lepromin test remains negative Therefore this method of cure does not depend on the development of a hypersensitization phenomenon It is suggested that in such instances the body has been able with the assistance of drugs to destroy large numbers of *M leprae* and an immunity analogous to natural immunity has developed It is interesting to note at this point that those cases which have been described as *persistent positive cases* that is those patients who remain positive to *M leprae* by routine methods of examination for periods of over four years sometimes show significant improvement and reach the stage of negativity when the drugs are combined with older methods for example intradermal injections of hydnocarpus oil This points back *to the suggestion that under these circumstances hydnocarpus oil may be* acting as an antigenic adjuvant The subject of immunity in leprosy therefore must be left at this point with the emphasis on the fact that a great deal of research of a fundamental and basic nature is needed before this complicated subject can be fully understood

V Diagnosis

It would not be within the scope of this article to give any detailed description of the methods of diagnosis of leprosy or do more than briefly mention the subject of differential diagnosis One must however stress in the introduction of a paragraph on diagnosis *that the lepromin test is not* a diagnostic test The very fact that a patient shows a lepromin positive *is no indication that the diagnosis of leprosy is confirmed* for there are many early lepromatous cases which are difficult to diagnose whose lepromin is completely negative On the other hand there is as is well known a certain amount of *cross immunity to leprosy and the presence* of other acid fast organisms for instance *M tuberculosis* will transform a negative lepromin into a positive It is also quite unknown whether other acid fast organisms such as the smegma bacillus which most if not all human individuals harbor in certain secretions also act in this way

And this may be one of the explanations of the spontaneous conversion of a negative lepromin to a positive in persons who never have been in contact with known infection The lepromin test therefore is of value only when a case has been definitely diagnosed for it is useful to the physician as it gives him confirmation as to the evolution of the disease in the clinical form which presents itself at the time of diagnosis and is confirmatory evidence along with clinical signs and the histopathological report of the strength of the tissue defense in an individual case

It is I think generally accepted that leprosy is primarily and initially a neural infection Khanolkar (1951) as already mentioned has pointed out that leprosy is neural in inception and one of the best aids to diagnosis in doubtful cases is a study in the changes in the nerve endings in the skin Weddell *et al* (1959) have shown that there are three neurohistological pictures in skin infected with *M lepræ* first the tuberculoid picture second the lepromatous and third the dimorphous It is impossible in an article of this nature to dwell on this important aspect of diagnosis One only can indicate that in tuberculoid leprosy the tissues of the dermis are grossly disorganized and the neural elements in the skin appear to be completely destroyed In lepromatous leprosy on the other hand there is seen a full complement of axons which are grouped in bundles—as they are in unaffected skin—but the bundles are surrounded by densely packed masses of cells (most of which contain acid fast bacilli) which fill the channels occupied by the neurovascular bundles in the normal skin Weddell *et al* (1959) state The neurohistological picture in established cases of lepromatous leprosy is consistent with local pressure on nerve bundles leading to relative ischemia of fibres and a minimal amount of nerve destruction followed by regeneration In established lepromatous leprosy acid fast bacilli are seen in profusion in all cells belonging to the reticulo endothelial system as well as in the Schwann cells and other neural elements In dimorphous lesions the pictures formed an intermediate series between those seen in established tuberculoid lesions and those seen in established lepromatous lesions but there is a consistent pattern which should be of diagnostic and prognostic significance in early cases A detailed study of the neurohistological pattern of the skin is of utmost importance in establishing a diagnosis of leprosy in doubtful cases

In discussing the diagnosis of leprosy it must be borne in mind that leprosy simulates a large number of dermatological and neurological conditions It may be necessary therefore to use special histological techniques with reference to nerve staining in order to confirm or reject a diagnosis of leprosy in those cases of doubtful diagnosis Weddell *et al* (1959) and Schofield (1959) have described these techniques

VI Classification and Signs and Symptoms

It may be considered that it is unusual to place classification before a description of the signs and symptoms of the disease but I have done this with deliberate intent because the subject has been a controversial one for many years It is important that the classification of leprosy should be a scientific one one that will give the person using it an appreciation as to the position the patient has reached in the development of the disease A classification is of little value if it is merely a catalogue of terms One need only review the history of classifications of other diseases for in stance diseases of the kidney and blood dyscrasias to appreciate this These classifications have become more and more complicated as these conditions were better understood

All too frequently classifications in leprosy can be likened to a series of compartments in which one files a name which one has given to a particular manifestation of leprosy rather in the manner of a post office clerk who places his mail in various pigeonholes such as London Liverpool New York Washington quite irrespective of the contents of the letters It was unfortunate that at the recent Tokyo Congress (1958) it was not possible to do more than outline a practical or field classification this however received the unanimous support of the Congress Nevertheless this classification is a strictly clinical one and is based on certain clinical signs and therefore is of little scientific significance In this instance leprosy is divided into macular cases with or without anesthesia in filtrated cases with or without anesthesia and cases whose only presenting symptom is anesthesia Generally speaking all cases which do not show anesthesia and belong to the macular group in which acid fast bacilli are not detected by routine methods of examination are placed into the inde terminate group Macular lesions that show anesthesia are classified as maculoanesthetic lesions The two polar types tuberculoid and leproma tous are retained in this classification and those infiltrated lesions that have characteristics of both lepromatous and tuberculoid leprosy are con sidered to belong to the borderline (dimorphous) group A place in the Tokyo classification for the pure anesthetic (pure neuritic or polyneuritic) case has been found and a separate group has been designated the neuritic group This classification is easy of application

It is of no real significance that the Latin American workers prefer to place the maculoanesthetic lesions as a subheading of tuberculoid leprosy and they are also in favor of placing the neuritic cases under the same subheading However if one looks at this classification from a detailed and critical point of view it is of very little help to the physician in esti mating prognosis or in endeavoring to find out just where in the scale of

the evolution of the disease a particular patient is Unfortunately the Tokyo Congress could come to no agreement with regard to a standard detailed or scientific classification and this matter has been left over to another Congress

It might however be of value here to discuss certain basic principles in approaching the classification of leprosy In the first place one should realize that the picture of leprosy is not a composite or stereotyped picture but a very variegated one It is necessary when one is considering leprosy to realize that there is a certain basic evolution and development of the disease and that it is our task in devising a classification to indicate as far as possible where a patient is in the development or evolution of the disease—in other words a classification should be symbolic of the ultimate prognosis Further in so classifying a case the very symbols used should be a guide to prognosis It may be said that once *M leprae* has entered the tissues and has begun to multiply there is constant warfare between the bacillus endeavoring to get the ascendancy and the tissues endeavoring to overcome the bacillus The former one might designate the lepromatous *element in leprosy* and the latter *one might call the* tuberculoid component In the great majority of instances the cases that present themselves are seldom completely lepromatous or completely tuberculoid and therefore in most if not all cases of leprosy there is evidence of both tuberculoid and lepromatous leprosy this phase through which Khanolkar and Cochrane (1958) believe all leprosy passes they have designated the dimorphous phase If in the course of the disease the tissues show a complete ascendancy then tuberculoid leprosy is seen On the other hand the presence of the bacilli in sufficient numbers results in the breaking down of the tissue response and the final evolution of the disease is then lepromatous leprosy Therefore the work of the Latin American writers in particular Pardo Castello and Tiant (1943) was essentially sound when they enunciated the polar conception of the disease

As pointed out elsewhere the confusion in respect to classification arises from the fact that the natural evolution of the disease in the human tissues has not been adequately studied It has been contended in this article that on entry of *M leprae* into the skin there is at first a minimal tissue response which histologically is seen in the form of a simple inflammatory reaction which consists of round cells with no characteristic distribution Without special techniques such as Weddell *et al* (1959) have described or the help of fluorescence it would be impossible to make a definite diagnosis of leprosy histopathologically This *indeterminate* phase however in most cases which progress is a passing one and sooner or later the tissue defense begins to declare itself This is seen by the

gradual transformation of the histopathological picture from one of a simple inflammatory reaction to a more definite and specific histology If there is inherent in the tissues a potential tissue immunity then the simple inflammatory reaction gives place to a histopathological picture which is indicative of the development of a defense that can be described as a tuberculoid response that is a concentration of round cells and lymphocytes around the appendages of the skin a commencing invasion of the nerves and the appearance of epithelioid or tuberculoid foci usually associated with the skin appendages If this tissue response is further stimulated either as a result of the natural development of the disease in the individual or by BCG vaccination then the tissues show a much greater capacity to put up an effective defense against the organism and definite and marked tuberculoid foci appear this is seen in a massive involvement of the whole corium with epithelioid cells and giant cells and with gross involvement and total destruction of the nerves in the subcutaneous tissue As a result the bacilli disappear from the tissues with subsequent healing of the lesion leaving not infrequently sequelae in the form of scars and signs of nerve damage as evidence of a successful battle of the tissues against this mycobacterial invader

On the other hand if for some reason the tissues cannot build up such a defense a completely different evolution is seen The simple inflammatory reaction is replaced by the mobilization of histiocytes or macrophages and these begin to phagocytose the bacilli—and spread them down and outward into the corium of the skin and thus the whole of the reticulo endothelial system is opened to attack In this way progressive lepromatous leprosy develops and there is no evidence whatever that the tissues can effectively combat this mycobacterial invader It is in this form of leprosy that bacteriostatic and particularly bacteriocidal drugs if available produce their most outstanding successes

The tissue response is not usually so clear cut as described for in the development of leprosy either as tuberculoid leprosy or lepromatous leprosy the individual usually passes through a phase or zone in which clinically and histopathologically there is evidence of both tuberculoid and lepromatous leprosy and this has been referred to as the dimorphous zone It is impossible to describe in the space available the detailed histopathological picture in the various forms of leprosy but Tables I and II give a brief summary of the clinical characteristics of the disease first in relation to macular leprosy (Figs 1–5) and second in relation to infiltrated leprosy (Figs 6–11)

TABLE I

CHARACTERISTIC FEATURES OF MACULES IN LEPROSY

Data	Maculoanesthetic[a]	Lepromatous	Dimorphous (indeterminate)
Size and number	Large, single or few in number	Multiple, small, and numerous	Initial large lesion and then numerous small satellite macules
Distribution	Characteristic and asymmetrical	Diffuse and symmetrical	May start as in tuberculoid macular leprosy showing a similar distribution, then smaller lesions appear; these are diffusely distributed as in leproma
Texture of skin	Rough and dry	Smooth and shiny	Tends to be rough and wrinkled or creased
Periphery	Definite	Vague	Large lesions show a more definite edge; that of the smaller lesions is indefinite
Sensation	Usually some loss of sensation; this may be relatively gross	Little or no loss of sensation	Variable; larger lesions tend to show loss of sensation and smaller lesions retain sensation
Bacilli	Usually negative	Except in the very early prelepromatous phase bacilli can always be demonstrated by standard methods of examination	Lesions are usually negative to standard methods of examination but may be positive occasionally and intermittently strongly so
Lepromin	Slightly positive or positive	Negative	Variable, never strongly positive

It is to be noted that the Latin American Workers (1959) consider that the maculoanesthetic lesion is essentially tuberculoid and that, therefore, they classify this clinical manifestation in leprosy along with tuberculoid leprosy and designate it tuberculoid macular.

TABLE II

SUMMARY OF THE CLINICAL CHARACTERISTICS OF INFILTRATED LESIONS

Data	Leprides (tuberculoid)	Dimorphous	Lepromatous
Size and number	Large and relatively few in number	Lesions numerous if there are large lesions there are also numerous smaller lesions scattered diffusely over the body	Lesions usually small individual lesion is difficult to differentiate because lesions tend to coalesce
Distribution	Characteristically asymmetrical on the outer aspect of extremities face scapulae and buttocks	Distribution as in lepromatous scattered all over the body tends to be symmetrical	Lesions scattered all over the body symmetrically and widespread in distribution
Edge and infiltration	Definite edge the infiltration slopes from the center to the periphery ending in a clear cut margin giving the lesions the shape of a saucer the right way up	Edge vague the infiltration slopes from the periphery to the center i e the most prominent part of the lesion or the part most easily seen is not the edge but toward the center giving the appearance of a saucer upside down	Seldom possible to differentiate the edge of an individual lesion for each lesion fades into the next except when nodules appear these are seen in the midst of the lepromatous infiltration
Surface	Rough frequently scaly may show healing center	Rough frequently scaly may show healing center but this *is less common*	Usually smooth unless there has been ulceration which leaves a *crust indicating* previous breakdown of the skin Lepromatous leprosy as it resolves may leave a tissue *paper appearance or wrinkling* of the skin when a nodule resolves there is an appearance as if a bubble has been pierced

TABLE II (Continued)

Data	Lepride (tuberculoid)	Dimorphous	Lepromatous
Sensation	Gross loss of sensation	Loss of sensation variable but in some lesions there is a similar gross loss	On the trunk sensation is variable but usually in the more marked forms of lepromatous leprosy sensation is absent in the extremities
Nerve	Greatly enlarged abscess formation fairly frequent	Enlarged abscess formation does not occur Occasionally cystic swellings are noted which are filled with gelatinous material but no pus	May be enlarged swollen and tender In late lepromatous leprosy nerves may be reduced to fibrous cords
Bacilli	Rare but if present demonstrable only for a short time (3 months)	Frequently numerous but may be scanty and lesions may remain positive for a considerable time	Enormous numbers
Lepromin	Strongly positive	Variable weak or even negative	Always negative

Figs 1–5 Macular lesions



Fig 1 Maculoanesthetic lesion There is a large macule just above the knee on the right side The section showed an epithelioid focus in the subcutaneous nerve



Fig 2 Maculoanesthetic lesions Note the multiplicity of the lesions their symmetrical distribution and the smaller ill defined lesions between the larger macules This is probably not a true maculoanesthetic case (that is a macular tuberculoid) but is in the dimorphous one however it is clinically maculoanesthetic

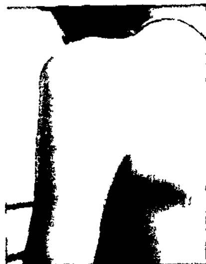


Fig 3 Prelepromatous macular lesions Note the vagueness of the edge of the lesions and the multiplicity of the lesions Under the Tokyo classification this group of lesions would have to be classified as indeterminate histologically and immunologically they are essentially of a lepromatous nature but insofar as no bacilli can be found by routine methods of examination they have to be placed in the prelepromatous category

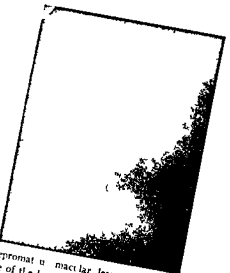


Fig 4 Lepromatous macular lesions There is a tendency to central clearing of the lesions and vagueness of the edge At this stage the macule becomes positive to routine methods of examination and therefore have to be classified as macular lepromatous leprosy

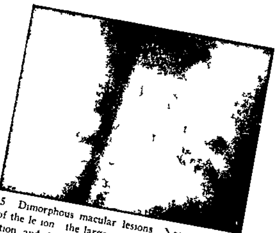


Fig 5 Dimorphous macular lesions Note the symmetrical nature of the lesion the large macules which tend to show loss of sensation and the smaller satellite macules appearing as outcrops from the larger macules These show no loss of sensation and the histological features manifest both tuberculoid and lepromatous features

Figs 6-11 Infiltrative lesions



Fig 6 Minor tuberculoid Note the single lesion on the outer aspect of the right cheek and the clear cut pebbled border Histologically this showed well marked epithelioid foci



Fig 7 Active major tuberculoid lesion Note the clear cut edge and that this is a single lesion The histology shows marked tuberculoid features and no evidence of a clear subepidermal zone.



Fig 8 *Reactional tuberculoid* Note the multiplicity of the lesions their symmetrical distribution and the clear cut edge of each lesion From Modern Trends in Dermatology (R M B MacKenna ed) p 161 (Fig 29) Hoeber New York 1954



Fi 9 Dimorphou infiltrative lep r y (typ al tubercul d) Note the multiplicity of th le ons their sym n try the vag w f th lge f a fe f the le n n l the outer p of mall infiltrat n fr m of th larg r pla ue



Fi 10 Dimorph us infiltrat ve lep r y (atypical lep m t u le i ns) Note th multiplicity of the le n the lapt t the larg l n n th right heck a d that th l ge f the nfiltration is from the periph ry to the cent r a the st prominent part of the le ion towar l th center a d not towar l th lge This l n sh a chara ter stic l rph a l tol gy w th a tr ng l pr utou el t



Fig 11 Nodular lepr y Notice the multiplicity of nodule o the arm and the ulceration of one of the nodule This patient had milar nodule scattered all o r the trunk and back

VII Therapy

Having briefly reviewed the present position of the classification of leprosy with particular reference to its clinical manifestations I pass on now to the present position with reference to therapy in the disease

It cannot be too strongly stressed that the general acceptance of di aminodiphenyl sulfone (DDS Dapsone) as the method of choice in the treatment of leprosy has resulted in a major revolution in the therapy of this disease and has brought relief to millions of sufferers who would otherwise be beyond hope This is not the place to review the steps which were taken *to develop the new treatment of leprosy except to say that* the pioneer work of Feldman *et al* (1941) and Faget *et al* (1943) in the United States of Brownlee (1948) and Francis and Spinks (1950) in Britain of Fourneau *et al* (1937) in France all contributed to the gradual elucidation of therapeutic problems of leprosy

Diaminodiphenyl sulfone was first administered parenterally in Madras in 1946 and Lowe (1950) realizing the possibilities of oral therapy with considerable courage began to give DDS by mouth In this connection a tribute is made to the late Dr John Lowe who did more than any other *person to develop the oral administration of DDS Hitherto this drug* had been considered too poisonous to be given by this means but as a result of his researches this new and powerful remedy has been made available to hundreds of thousands of persons who suffer from leprosy

The regimen of dosages of both parenteral DDS and oral DDS has been worked out satisfactorily but the general principle in any regimen of dosages is to start at a low dose and gradually increase for it is the gradual induction of the sulfones that results in the remedy being tolerated For detailed reference to the treatment of leprosy with DDS and its derivatives the reader is referred to the numerous papers published on this subject

It should however be mentioned that in countries where patients with leprosy tend to show a maximum intolerance to the drug the average standard dosage tends to be too high and it is recommended under these circumstances that the following be taken as the safest method in applying the therapy It is suggested that the starting dose be 25 mg of DDS twice a week at the end of 14 days the dosage is increased to 50 mg twice a week then at 14 day intervals it is increased by 25 mg increments until 100 mg twice a week is reached The dose is then raised by 100 mg every month until 300 mg twice a week is given When this dosage is reached if more convenient one 100-mg tablet may be given every day omitting Sundays If the method of choice is parenteral injection either deep subcutaneously or intramuscularly the drug can be conveniently

suspended in a suitable medium—coconut oil groundnut oil or ethyl esters of hydnocarpus oil—in the strength of 200 mg per cubic centimeter

Even though the greatest care is taken there are always a certain number of patients who under no circumstances can tolerate the parent sulfone This is more often seen as mentioned previously in the Caucasian and Mongolian races and particularly applies to the active moderately advanced lepromatous case and to certain forms of the infiltrated dimorphous group Under these circumstances therefore an alternative to the parent sulfone has to be sought and the writer believes that the first choice of an alternative remedy is parenteral Sulphetrone administered in a 50% aqueous solution intramuscularly or deep subcutaneously It has been shown by Cochrane (1959a) that as low a dose as 1 gm (1 cc twice a week) and by Browne (personal communication) 1 5 gm (3 cc once a week) are effective therapeutic dosages at this level of dosage the cost of Sulphetrone is only slightly higher than that of DDS given by mouth and certainly is no more expensive than DDS given parenterally If the commencing dose is not more than 0 1 cc twice a week and the dosage is increased by 0 1 cc every fortnight until 1 cc twice a week is reached it will be found that comparatively few persons are intolerant to sulfone therapy With careful treatment by parenteral injections of a 50% aqueous Sulphetrone the writer has never seen any signs of gross anemia hepatitis or psychosis and he considers that this remedy is free from major toxic complications

When discussing the therapy of leprosy it should be mentioned that in the United States and in Latin American countries Promin and Diasone still are in favor as the routine treatment for leprosy The starting dose of Promin usually recommended is 2 gm in 5 cc of distilled water giving the drug daily The dose then is increased after 1 or 2 weeks by 1 cc until a daily dose of 12 5 cc is reached The dosage for children depend on age weight general physique and individual tolerance The drug should be administered daily for from 1 to 3 months followed by a rest period of from 1 to 2 weeks after which treatment is resumed The dosage and the length of the rest periods may be modified in accordance with the requirements of the patient There is some evidence that Promin is effective in a much smaller dosage than that originally advocated

When Diasone is preferred the following dosage is suggested

1st week	0 3 gm (1 tablet)	daily for 6 days	1 day of rest
2nd week	0 6 gm (2 tablets)	daily for 6 days	1 day of rest
3rd week	0 9 gm (3 tablets)	daily for 6 days	1 day of rest

The maximum dose recommended for the adult is three tablets per day (0 9 gm) As *one third of the molecule of sulfoxone* sodium is hydrolyzed to DDS when given by mouth this dosage over a period of 6 days would be 1 8 gm per week or almost the equivalent of 2 gm (2000 mg) of the parent sulfone This in my opinion is too high A maximum of not more than two tablets a day need be given In many instances such as in severe lepromatous cases or for adult persons of slender build (140 pounds) one tablet a day 6 days a week should suffice

VIII Reactional States and Their Treatment

There has been a great deal of confusion with reference to the definition and description of reactions in leprosy I think the best and clearest way to approach this subject is to subdivide as follows the reactional states as those (1) due to a bacterial allergy (2) due to exacerbation and progression of the disease (3) of a peculiar nature which are rare and unusual (4) in tuberculoid leprosy (5) in dimorphous (or borderline) leprosy

A States Due to Bacterial Allergy

These conditions occur only in lepromatous leprosy and have been described under the general names erythema nodosum leprosum panniculitis nodosum and erythema induratum leprosum This reactional phase of leprosy may show itself in a sudden attack or may be gradual in its onset The patient may complain of malaise and some headache and if the temperature is taken it is found that there is a definite variation of the normal rhythm of temperature although the fever is not actually registerable As the reaction progresses fever is manifest and sometimes it is as high as 104 or 105 F Along with the fever and frequently before its actual onset appear the characteristic lesions of erythema nodosum These are rose spot nodules which tend to be intracutaneous rather than subcutaneous and measure from 2 mm up to as much as 2 cm in diameter In the acute phase the individual rose spot nodules usually last not more than 24 hours to 48 hours After an attack of erythema nodosum there may be no visible sequelae but more usually the result of the attack is to leave a purplish stain and sometimes definite desquamation of the skin In the more severe phases of erythema nodosum the lesions are much larger leaving a residual deep purple stain which may remain for several months and with evidence of desquamation A graver form of erythema nodosum has been described as erythema induratum leprosum Most authorities I think accept the fact that erythema nodosum occurs only *in lepromatous leprosy* and *is probably of the* nature of a

bacterial allergy Some persons consider that it may be analogous to the Herxheimer phenomenon, which used to be seen in the treatment of syphilis when arsenical preparations were used

The treatment of erythema nodosum is along the usual lines The specific drug should be stopped at once general treatment applied and if the temperature does not subside within 4 days—during which time a general examination and investigation of concomitant conditions can be undertaken—then more vigorous treatment must be given Most authorities recommend that before cortisone is used for this reaction a course of tartar emetic or one of the trivalent antimony products should be given If these measures fail and erythema nodosum continues to be distressing cortisone should be given Some persons give a high dosage of cortisone for a few days and then slowly tail off the drug others recommend small dosages of cortisone building up to a dosage that just holds the erythema nodosum but may not suppress it altogether For details of this treatment the reader is referred to standard textbooks on leprosy

B States Due to Exacerbation and Progression of the Disease

These are very serious conditions and generally speaking cortisone is not indicated unless as a result of continuous reactions the patient is in great distress running a high fever with fresh lesions appearing and the older lesions breaking down Treatment of chronic reactional states will tax the ingenuity of the physician considerably and patients suffering from this complication should be hospitalized and brought under specialist treatment. It is for this condition that a 1% solution of Mercurochrome in a dosage of 3–5 cc intravenously every other day may be found useful Cortisone should be withheld unless there is no other means by which to hold the reaction and the disease is progressing and the patient is in acute distress Once cortisone therapy has been commenced then it may be necessary to continue this therapy for a considerable time even up to two years

C Unusual States of a Peculiar Nature

1 *Acute Pitting Edema*

This has been described by Davison (1949) as leprotic elephantiasis It may occur during an acute phase of leprosy sometimes associated with erythema nodosum and at other times associated with the more progressive forms of reaction in which the hands and feet become hot and tender and very swollen and pit on pressure Systematic treatment is applied and the general treatment of reactions with cortisone is given only if this condition is complicated with such signs as erythema nodosum.

Elevation of the bed careful continuation of sulfone therapy and general methods will usually bring great *amelioration of the condition* The condition of acute pitting edema in leprosy must be differentiated from the long standing stasis which occurs in advanced lepromatous cases and which is chronic and is not a reactional phenomenon

2 The Lucio Phenomenon

This has been well described by Latapi and Zamora (1948) and is almost entirely confined to the South American continent and to Mexico The following points may help the reader to understand if occasion arises how to manage this serious complication of leprosy The Lucio phenomenon occurs only in that form of the disease known as the diffuse lepromatosis described by South American writers and it is particularly to be noted that there is a generalized diffuse infiltration of the skin *without* any visible infiltration or corrugations It is on this smooth rather greasy skin that the particular lesions appear that have been described by Latapi and Zamora (1948) as follows first there are only a few lesions then they occur in small outbreaks (brotes) and with the passage of time and after repeated acute eruptions or stages the lesions become more numerous and important They predominate on the extremities and only in the advanced cases are they widely disseminated In number size and shape these lesions are variable often being triangular or irregular they average fifteen days in duration At first the lesion is pinkish ill defined and painful and sometimes it is infiltrated A few days later it shows a darker center which does not disappear under pressure This center soon approaches the surface and there forms a small very thin dry brown scab which *finally drops off to leave an insignificant scar* In larger and more inflammatory elements there is formed a dark flaccid blister which bursts leaving a deep ulceration with jagged edges surrounded *by an inflammatory zone In addition particularly* on the legs there are secondary pyodermic lesions and chronic cellulitis which complicate the condition

Davison (1959) recently has suggested that there is a modification of the Lucio phenomenon seen in South Africa and that this phenomenon appears as a sudden crop of blebs one or more in number occurring only on the body and the limbs The fluid in the blebs is at first translucent but later becomes blackened the blebs break to form deep ulcers and the residual scars deepen and usually give the appearance of crushed tissue paper Unlike the true Lucio phenomenon in these cases the prognosis is good provided the secondary infection is controlled by means of antibiotics while the leprosy is being treated by sulfones Whether this

condition is analogous to the Lucio phenomenon it is impossible to state without further information and investigation The acute condition is particularly distressing when complicated with neuritis Davison (1959) recommends ACTH rather than the more potent cortisone derivatives and suggests that the dosage should be 5 units daily for 7 days and then if necessary 10 units daily for a further 7 days Treatment can be stopped as soon as the pain disappears He states however that if the case is not responding to ACTH prednisone (10 mg twice daily) should be given In addition he suggests that the following drugs are of value Butazolidine vitamin B_1 and vitamin B_1 He states that in his ex perience three 0 1 gm tablets of Butazolidine daily for 7 days then two tablets for the next 7 days and then one tablet daily until relief is obtained is a suitable dosage In his opinion this is not a dangerous dose of Butazolidine and does not give rise to toxic signs

D In Tuberculoid Leprosy

It should be noted that reactional states in this form of leprosy and in the dimorphous group are evidences of a tissue response or a tissue hypersensitivity There are three forms of reaction in tuberculoid leprosy which must be distinguished (1) tuberculoid leprosy in reaction (2) re actional tuberculoid leprosy and finally (3) a form of tuberculoid leprosy which has been described by Dubois as *Poussée bacillifère* These three states have to be dealt with separately as the approach to their management is entirely different

1 Tuberculoid Leprosy in Reaction

This is a localized form of tissue sensitivity It occurs in what one may call the established tuberculoid case—that is in that type of tuberculoid leprosy in which the lesions are few in number with clear cut and definite edges and asymmetrical in their distribution In this form of reaction if the patient shows more than one lesion all the lesions do not neces sarily show a reaction phenomenon but one lesion for instance a lesion on the face may pass into acute reaction and show itself in the form of edema erythema desquamation and even ulceration The lesion itself may become acutely tender and nerves to the lesion may be swollen en larged and tender Generally speaking all that is necessary is to stop the sulfone medication and the reaction according to its severity gradually passes off The more severe and acute the reaction the more quickly does it subside one should mention at this point that when a lesion of tuberculoid leprosy on the face passes into an acute phase of reaction facial paralysis may be one of the unfortunate sequelae It is the opinion

of the writer that whenever a tuberculoid lesion begins to show erythema and desquamation all sulfone medication should be stopped for the reactive phenomena in tuberculoid leprosy always subside and the lesion retrogresses but the possibility of severe nerve damage in the form of facial paralysis claw hand or drop foot cannot be ignored This type of *tuberculoid reaction is confined to areas in which the lesions show activity*

2 Reactional Tuberculoid

The difference between *reactional tuberculoid leprosy and tuberculoid* leprosy in reaction is as follows In the established tuberculoid case the lesions are few have clear cut margins and tend to be asymmetrical when they go into reaction all lesions do not pass into reaction simultaneously On the other hand in reactional tuberculoid lesions the lesions are many the edges of the lesions are clear-cut the distribution is symmetrical when these cases go into reaction all the lesions react simultaneously Therefore reactional tuberculoid is a more serious condition than tuberculoid leprosy in reaction and is generally speaking a mutilating condition because not only do the lesions show intense reaction in the form of edema desquamation and possibly even ulceration—Ryrie (1938) used to refer to some of these cases as ulcerating tuberculoid—but along with the activity of lesions gross damage of the nerves is inevitable *Therefore if a case shows any tendency to reactional phenomenon in* this type of leprosy all sulfone therapy must be stopped at once and no sulfones given until the reaction has subsided Reactions are treated symptomatically Cortisone has been suggested for these cases but I have never been able to convince myself that this is either desirable or beneficial If cortisone is given then it must be given in very large doses and over a very short time

3 Poussee Bacillifere

This is a form of tuberculoid reaction that is seen more frequently on the African continent than anywhere else and *shows itself as a localized* tuberculoid lesion with a papulated or pebbled border Generally speaking these lesions are quiescent and show very little activity but every *now and then they* pass into a phase of reaction when the small papules become succulent and erythematous and during this stage numerous acid fast bacilli may be found by routine methods of examination. There is also a tendency for these lesions to spread centrifugally and cover a large area of the body surface e g the arm the leg or the back Again treatment in this condition is to stop all sulfone remedies The reaction is not a serious one except that during the reactive phase of this type of

tuberculoid leprosy the lesions as already mentioned become highly positive and the person is infective during the period of the reaction

E Reactional Dimorphous Leprosy

Ryrie (1938) many years ago referred to this reactional phenomenon as reactional tuberculoid It is one of the most severe types of reaction that can be seen in leprosy and is always of very serious import—serious for two reasons first because the reaction is very severe the lesions becoming erythematous edematous and not infrequently ulcerating second in association with reaction there is always evidence of considerable nerve damage and the sequel of the reactional dimorphous lesions usually is gross disability Furthermore when the reaction subsides the patient is in an unfavorable immunological condition and is liable to pass into lepromatous leprosy As soon as the reaction has subsided the patient becomes emaciated and weak and unless carefully nursed may even die as the result of the emaciation which supervenes because of the severity of the reaction Treatment for this condition is to stop sulfone therapy immediately to treat the condition symptomatically and to take every measure possible to prevent deformity this means judicious splinting and careful exercise and passive movements as the reaction subsides Therapy of any kind except symptomatic therapy should be withdrawn entirely

One cannot close a note on the reactional phenomena in leprosy without mentioning what the Japanese have called acute lepromatous infiltration (*akuter Schub*) In this condition the lesions become grossly infiltrated and edematous and sometimes the reaction is so severe that it simulates a cellulitis The writer is personally of opinion that the phenomenon of *akuter Schub* is an atypical lepromatous reaction and should probably be included among the reactional phenomena in dimorphous leprosy Some of the lesions described by Japanese workers may fit into the description which has been given of reactional tuberculoid leprosy but whatever the condition is it behaves in a way which is not typical of lepromatous leprosy The treatment for this is to stop all sulfone therapy and to treat the cases symptomatically

F Therapeutic Remedies Advised after Reactional Phenomena Have Subsided

There is a general rule that if severe reactional states have been induced by DDS patients should be given one of the alternative remedies as follows (1) parenteral Sulphetrone (2) CIBA 1906 (diphenylthiourea) or (3) thiosemicarbazone

1 *Parenteral Sulphetrone*

Personally the writer finds that in most cases the patient will tolerate small doses of aqueous Sulphetrone when he will not tolerate any form of DDS and that the commencing dosage of aqueous Sulphetrone must be very small 0 1 cc subcutaneously twice a week increasing by 0 1 cc every week until 1 cc is reached *Sometimes even this regimen of dosage* is too rapid and therefore one must adjust the increase of dosage according to the severity of the reaction

2 *CIBA 1906 (Diphenylthiourea)*

This remedy has recently been introduced in the treatment of leprosy and is of value in those cases that show intolerance to all forms of sulfone therapy The commencing dose advocated in reactive states is 250 mg or a quarter tablet a day increasing every week by 250 mg until 2 gm or four tablets a day are given Generally speaking it may be said that with the better understanding of the mechanism of reactions with the judicious use of cortisone and the subsequent careful choosing of the specific remedy the treatment of reactional phenomena is much more satisfactory today

3 *Thiosemicarbazone*

Many leprologists advocate thiosemicarbazone as an alternative remedy when a patient has shown intolerance to DDS by the precipitation of a reactional phenomenon This drug has been advocated especially in cases of neuritis Under these conditions the commencing dosage is 25 mg a day increasing by 25 mg every fortnight until 100 mg a day is given The writer considers that this is the optimum dose of thiosemicarbazone and does not advocate an increase to more than 150 mg a day

The great advantage of diphenylthiourea (DPT) is that it has no relationship whatever to any form of sulfone and therefore is a completely new weapon of attack against *M leprae* Unfortunately there are two drawbacks to the drug One is its price and the other is the relatively large dosages which have to be given daily therefore it is generally speaking unsuitable for outpatient treatment and quite unsuitable for mass therapy On the other hand in those individual cases who have shown intolerance to DDS either by the precipitation of severe reaction or because of drug toxicity changing over to DPT has provided the physician with a remedy that enables him to continue the treatment of leprosy as successfully as though he had used the sulfone drugs and the patient had not been in reaction On the other hand because the basic structural formula of DPT is very similar to that of thiosemicarbazone there is a tendency for resistance to form after prolonged medication with

this drug Davey (1959a b) has indicated that after three and a half to four years there is some evidence of resistance DPT therefore should be used for a period of not more than two to two and a half years After that period it will probably be found that the disease has been sufficiently controlled for the patient to be put back on to one of the sulfone preparations

IX Newer Drugs in the Therapy of Leprosy

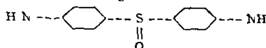
In the last year three new drugs have been mentioned in connection with therapy in leprosy probably the most interesting is that known as Etisul (diethyldithiolisophthalate) The drug was first reported by Davey (1959b) his initial reports were very striking in that the bacteriologic index of lepromatous cases fell almost to zero within a few weeks of application of the drug The drug is given by inunction and its greatest drawback is that it causes a garliclike odor of the breath since it is partly excreted through the lungs

There are several curious features of Etisul in relation to its therapeutic action First it is said that the bacilli disappear—but the granuloma in the skin still remains! In other words clinical improvement lags behind bacteriological improvement It is suggested that the drug acts as a bactericidal agent and that this explains its peculiar action but one has to ask the question If the bacilli are killed as rapidly as apparently is the case why is it that erythema nodosum has not been reported on its administration This drug however opens a new approach to the therapy of leprosy for it may be possible to produce a marked reduction in the number of bacilli in lepromatous cases and then to follow up this treatment by one of the known and potent antileprosy drugs such as DDS Sulphetrone or DPT Further experience has shown that treatment with Etisul should be reserved for the hitherto untreated lepromatous case and should be in the nature of intensive therapy for 3 to 6 months and should be combined if possible with DPT or sulfone therapy

Very recently Schneider *et al* (1959) have reported on the treatment of leprosy by a new sulfonamide sulfamethopyrazine known also as Kynex (Lederle) and Sultirene (Specia) Preliminary reports on this sulfonamide appear to be encouraging I had the privilege of seeing some of the photographs that Dr Languillon had with him at a World Health Organization Conference held for leprosy workers in the area south of the Sahara in Brazzaville in April 1959 All one can say at the present time is that these initial reports appear to be encouraging and that this drug deserves a further *extended trial*

A third drug I would like to mention diaminodiphenylsulfoxide was

first studied by Buu Hoi *et al* (1955) It is a derivative of diaminodi phenylsulfone and has the following structural formula



Davey s (1959b) conclusion with reference to this drug is as follows Here then is an active drug comparable in activity with DDS like it suitable for treatment either daily or twice weekly and on a basis of 18 months experience having little toxic action and little tendency to evoke complications It is not a proprietary preparation These good qualities commend its further study

X Prophylaxis

I cannot close without reviewing briefly the present position with regard to the prevention of leprosy It may be said that there are three schools of thought with reference to the approach of public health authorities to the organization of preventive schemes to control the spread of the disease The French authorities particularly those in French Equatorial Africa and French West Africa as represented by General Pierre Richet advocate the organization of mass antileprosy campaigns by a method known as periodic circuits of sulfonic treatment This involves the extensive use of landrovers bicycles or in the more inaccessible areas horseback riding and even camels As a result of the advocacy of this method of mass campaigns there has been an admirable and encouraging antileprosy organization created throughout French Equatorial Africa and French West Africa It is too soon to say what degree of ultimate success will be achieved but there has been a great deal of effort put into these campaigns resulting in a great increase in the number of cases of leprosv treated and in reducing the fear of the population toward leprosy This is a very remarkable achievement In the opinion of the writer the work of the French African authorities is highly commendable but tends to depend too much on four factors namely the enthusiasm of the staff in undertaking what is a colossal task the possibility of super vising adequately the various centers where leprosy treatment is given the maintenance of accurate and complicated filing and records and lastly the maintenance of the enthusiasm of the population for taking oral remedies or presenting themselves for injections regularly over a period of months and years Therefore although the French African authorities are to be congratulated on their work we should compare this method of organizing a preventive campaign with those adopted in Nigeria and in East Africa with considerable success

This method was put into effect by Kinnear Brown (1934) in Eastern Nigeria then the work was continued by Davey *et al* (1956) and resulted in a great reduction in the incidence of leprosy Kinnear Brown has followed similar methods in Uganda namely (1) the organization of treatment villages (2) the organization of leprosy sanatoria and (3) supplementary outpatient clinics This approach to the problem of the prevention of leprosy seems to the writer to be the most fruitful method by which leprosy can be controlled in a territory In the first place it accepts the principle which I think should never be forgotten that the successful control of leprosy depends on some form of isolation in which the open or infective case is prevented from coming into close contact with healthy members of the community Second it provides a group of sanatoria whose staff can act as consultants to the leprosy treatment centers and affords means by which cases needing special attention or hospitalization can be taken care of Third it encourages the formation of outpatient clinics organized and administered by the general health and medical service of the country and so begins the important task of integrating the leprosy campaign into the general health service There is however a third method of prevention of leprosy exemplified by the system in vogue in South Africa—that of limited compulsory segregation requiring all infective cases to be isolated in a leprosy sanatorium or a colony and not discharging such cases until they are non infective

These three methods have their advocates but their success depends *on the stage of the epidemic the pattern of the disease in a community* and the economic development of the country In South Africa where general social standards and standards of living are by and large very much better than in other parts of Africa the situation may be comparable to that seen in Norway eighty or more years ago where a limited but common sense method of segregation was put into force and as a result of a more enlightened attitude toward the leprosy patient the number of cases fell from about 3000 in 1896 to practically none in 1959 The total number of cases at the present time in Norway is I believe less than a dozen It is true however that no form of segregation nor any form of widespread therapy by mobile teams could possibly hope to succeed without an effective therapeutic agent The present methods of treatment by the sulfone preparations give hope that these methods applied intelligently and persistently will in the course of time reduce the incidence of leprosy in the endemic countries to a point when it can be said to be virtually controlled

It must however be pointed out that conclusions cannot be drawn

from isolated experiments for there are too many factors influencing the pattern of the epidemic of the disease to justify the statement that one method is superior to another It is certain however that a combination of reasonable outpatient treatment with the cooperation of the indigenous population to develop treatment villages along with the use of leprosy sanatoria for instruction for training for research and for hospitalization of the needy case is the principle on which leprosy campaigns should be organized

Finally a word must be said with reference to the prophylactic administration of sulfones to the contacts of lepromatous cases and to children and also with reference to the extended use of BCG vaccination There are advocates of widespread prophylactic sulfone medication but it must be pointed out that this method of prophylaxis is based on the theory that sulfone therapy is a sure and effective way of preventing the bacilli from multiplying and ultimately of causing them to disappear from the tissues There is also a certain amount of evidence to show that sulfone therapy has some effect in the prophylaxis of the disease We know too little however about the possibility of drug resistance to risk such an eventuality arising All the present evidence goes to show that so long as DDS is administered to a patient bacilli are reduced and suppressed If however the treatment is not prolonged for a sufficiently long time or other factors supervene which result in this method being ineffective the bacilli begin to reappear in the tissues Therefore it is the writer s opinion that unless a child is in constant contact with a lepromatous case and there is no means by which it can be separated from its source of infection there are no sound reasons for giving it prophylactic sulfone therapy All methods of prophylactic sulfone therapy should be based on a carefully organized experiment extending over a period of ten to fifteen years for there is no other way to verify statements that prophylactic sulfone therapy is an effective means of preventing the further spread of the disease in the community

Widespread BCG vaccination for the control of leprosy is a method which is still unproved and it would appear to me to be illogical to undertake this measure without adequate controls and the organization of a scientific and statistically planned experiment Admittedly if there is BCG vaccination in connection with a campaign against tuberculosis the possibility of BCG vaccination stimulating a favorable tissue response in leprosy should be considered and such a campaign should take note of populations in which there is a relatively high endemicity of leprosy

XI Summary and Conclusion

An attempt has been made to review briefly the present position with regard to leprosy and to summarize so far as possible modern trends and opinions in regard to diagnosis and classification the effectiveness of therapy the present position in regard to immunology and the various methods advocated in the control of the disease Today leprosy is receiving attention which it never has had before and there is an increasingly scientific approach to the disease this augurs well for our understanding of this ancient disease and is sure to result in better and more effective methods of control

I must remind readers of this review that the traditional attitude toward leprosy must be basically altered and that one must remember that to understand leprosy is to understand medicine The effective and successful treatment of leprosy presupposes a thorough appreciation of the general principles of therapy particularly as they apply to chemotherapeutic and antibiotic remedies Organization of adequate antileprosy campaigns needs better understanding of the epidemiology and immunology of the disease In other words leprosy touches almost every scientific discipline and will be fully understood only when the medical profession in general and particularly those members of the medical profession whose task it is to teach undergraduates and postgraduates realize that leprosy *is medicine* and *not* just a social problem that can be relegated to the paramedical personnel and to the few persons who out of altruistic philanthropic or religious zeal see a vocation in this work Vision and professional competence are essential if we are to combat leprosy adequately and establish confidence between ourselves and the public

In closing one would like to emphasize the need for the creation of a world center for the study of leprosy where senior leprologists can meet and exchange opinions where postgraduate students can appreciate leprosy in its world setting and where research workers can gather information that will help them in coordinating their basic research to certain aspects of leprosy in brief a powerhouse for the stimulation of research teaching and training in leprosy throughout the world a central registry of histopathology and a reference laboratory where the variegated pattern of leprosy can be appreciated For in the opinion of the writer all campaigns with regard to leprosy all understanding of the disease in particular countries must be placed against the background of the world pattern of the disease

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The Multiple Facets of *Entamoeba histolytica* Infection

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Experience modifies human beliefs To make the best possible use of experience is one of the great human tasks and to work for this task is the proper vocation of scientists G Polya[1]

I Introduction

From 1875 when *Entamoeba histolytica* was discovered in a Russian patient with fulminating amebic colitis until the present time this protozoon has intrigued and stimulated the investigative efforts of parasitologists and clinicians alike in attempts to explain its host parasite relationship and significance As a result of these studies a wealth of valuable information has been accumulated but as in other scientific areas students of amebiasis differ most in their ability to see through the mass of data and construct a reasonable hypothesis that will satisfy the multiple observations (Villee *et al* 1958) It will be the purpose of this review to show that *E histolytica* exhibits a great variety of characteristics as a result of its intrinsic properties and the many natural environments in which it is found as well as under the experimental conditions to which it has been subjected

II Historical Landmarks

Most of the important pioneer contributions to our knowledge of *Entamoeba histolytica* have been often recorded in the medical and

[1] Introduction and Analogy in Mathematics Vol I p 3 Princeton Univ Press Princeton New Jersey 1954

parasitologic literature and are mentioned in this paper only for purpose of orientation Lambl (1860) in Prague first reported amebas in human excreta but he apparently overlooked their potential significance in his enthusiasm to incriminate the duodenal flagellate *Giardia lamblia* as an agent of intestinal disturbance Lewis (1870) and Cunningham (1871) in India and later Grassi (1879) and others in Italy also found amebae in the stools of sick and healthy persons but doubted the clinical importance of these microorganisms In 1875 F Losch a clinical assistant in St Petersburg discovered motile amebae with ingested red blood cells in the dysenteric stools of a patient and later at autopsy of the patient detected colonic ulcers containing active amebae He fed and introduced per rectum some of this dysenteric menstruum into four dogs one of which developed dysentery and when sacrificed exhibited colonic lesions with amebae similar to those seen post mortem in his patient However Losch failed to appreciate the importance of his observations On the other hand Koch and Gaffky (1887) Kartulis (1886 1887, 1891 1904) and Kruse and Pasquale (1893) in Egypt Hlava (1887) in Prague Osler (1890) Stengel (1890) and Musser and Dock (Dock 1891) in Philadelphia Kovacs (1892) in Budapest and Richard Strong (1901) in the Philippines in cases of dysentery and in some instances also abscess of the liver and brain demonstrated that these pathologic states were due to invasion by this ameba Meanwhile Councilman and Lafleur (1891) in Baltimore published their monographic study on amebic colitis

Casagrandi and Barbagallo Rapisardi (1895) created the genus *Entamoeba* for *Amoeba coli* of Grassi (1879) Strong (1901) distinguished this species clinically from the dysentery producing ameba and also by experimental tests in kittens demonstrated that the former species was nonpathogenic and that the latter species which he referred to as *Amoeba dysenteriae* was the cause of amebic colitis In 1903 Schaudinn renamed this disease producing species *Entamoeba histolytica* For nearly two decades there was considerable confusion concerning the cystic stage of this ameba Earlier investigators of acute amebic infections confined their attention to the motile trophozoite Quincke and Roos (1893) observed the cystic stage but failed to give an adequate description of the number and specific characteristics of the nuclei In 1903 first Huber then Schaudinn provided these data showing that cysts of *E histolytica* were morphologically distinguishable from those of *E coli* However Viereck (1907) working in India considered the four nucleate cysts of *Entamoeba* to belong to a new species which he designated as *E tetragena* and Max Hartmann (1908) in South Africa adopted this same name for cysts which he had called *E africana* a year earlier (Hartmann 1907)

In his Philippine studies Strong (1901) distinguished between two etiologically and clinically different types of dysentery the one due to infection with *E histolytica* the other caused by the enteric bacillus which had been discovered by Shiga in 1896 (1898) and by Flexner (1900)

Several of the earlier investigators employed dogs and kittens as experimental test animals to demonstrate the pathogenicity of the amebae recovered from their clinical cases Losch (1875) Hlava (1887) Kruse and Pasquale (1894) and Harris (1901) employed dogs whereas Hlava (1887) Kartulis (1891) Kovacs (1892) Quincke and Roos (1893) Huber (1903) Kruse and Pasquale (1893) Marchoux (1899) and Strong (1901) utilized kittens possibly because these animals developed acute amebic colitis more rapidly than dogs Harris (1901) first obtained liver abscess in young dogs following intrarectal inoculation of trophozoites of *E histolytica* Similar techniques with kittens performed by Marchoux (1899) Craig (1905) and several later workers resulted in secondary amebic lesions in the liver

Successful experimental human infection with *E histolytica* was first reported by Musgrave and Clegg (1905) in the Philippines in a single previously uninfected volunteer who was given a gelatin capsule containing a 3 week-old ameba culture of material obtained from a case of dysentery The classic experiment with human volunteers was performed in Manila by Walker and Sellards (1913) who fed cysts of *E histolytica* from healthy carriers to twenty uninfected inmates of Bilibid Prison Eighteen developed *E histolytica* infection of whom four exhibited dysenteric stools Other volunteers who ingested cysts of *E coli* acquired symptomless infection with this ameba This experiment provided conclusive evidence that *E histolytica* has the ability to produce symptoms whereas *E coli* is a harmless parasite

Although much valuable information was obtained from the clinical and pathologic study of human infections with *E histolytica* and was materially supplemented by similar studies on experimentally infected susceptible animals a new horizon of investigation was opened with the discovery of methods for its *in vitro* cultivation Cutler (1918) is usually regarded as the person who first succeeded in this attempt but the first reproducible technique was provided by Boeck and Drbohlav (1925) who developed two diphasic media the Locke Egg Serum (LES) and Locke Egg Albumin (LEA) both with a solid base and a liquid overlay without starch Craig (1926) devised a monophasic medium consisting of inactivated serum in Locke s solution also without starch However Dobell and Laidlaw (1926) and practically all subsequent workers irrespective of whether they modified the earlier culture media or developed

new techniques added starch grains to the liquid phase to enable the ameba to grow and multiply more rapidly The most comprehensive studies resulting from *in vitro* cultivation of *E histolytica* and other intestinal amebae were those of Dobell (1928 1931) whose investigations constitute a volume of information on the life cycles of these protozoa

III Nomenclature of *E histolytica*

Remarkable confusion has existed with respect to both generic and specific names of the ameba which has the potential of tissue invasion and disease production in man Originally Losch (1875) referred to it as Amoeba coli Councilman and Lafleur (1891) as Amoeba dysenteriae in both instances without reference to zoological nomenclature and hence without italicizing the generic and specific names similarly Kovacs (1892) used both specific designations previously employed without italics Casagrandi and Barbagallo Rapisardi (1895) created the genus *Entamoeba* for the nonpathogenic species *coli* which was accepted by Schaudinn (1903) who included the pathogenic species in this genus under a newly created species name *histolytica* Additional species designations which have been employed include *coli* var *tetragena* Viereck 1907 *africana* Hartmann 1907 *tetragena* Hartmann 1908 *minuta* Elmassian 1909 *nipponica* Koidzumi 1909 *hartmanni* Prowazek 1912 *brasiliensis* Aragão 1912 *venaticum* Darling 1915 *tenuis* Kuenen and Swellengrebel 1917 *minutissima* Brug 1917 *coli communis* Knowles and Cole 1917 *paradysenterica* Chatterjee 1920 *sinensis* Faust 1923 *falcata* Kofoid and Swezy 1924 and *dispar* Brumpt 1925 Some of these names resulted from the belief that variations in morphology of the trophozoite or cystic stage of the organism observed justified the creation of a new name In some instances geographic location was at least partly responsible and in others evidence of pathogenic capacity was the essential reason Wenyon stated (1926 p 185) If Schaudinn had recognized the fact that the amoeba which Losch called Amoeba coli was the pathogenic amoeba and had given it the name *Entamoeba coli* endless confusion would have been avoided but as the matter stands at present there seems to be no alternative unless further confusion is to be caused but to retain Schaudinn s *E histolytica* for the pathogenic form and *E coli* for the non pathogenic one

Generically the difficulty has not been so great it has been due primarily to a failure to distinguish between the genera *Entamoeba* Casagrandi and Barbagallo 1895 and *Endamoeba* Leidy 1879 which was created for the species *blattae* Butschli 1878 a common intestinal ameba of cockroaches (*Periplaneta* spp) On September 19 1928 the International

Commission on Zoological Nomenclature ruled that the former generic name is synonymous with *Endamoeba* and hence must be superseded by the latter Many protozoologists and clinicians particularly in the United States in an attempt to conform to the Commission s Opinion used the generic designation *Endamoeba* even though they may have doubted its validity On the other hand a considerable group of protozoologists were convinced that *Endamoeba* Leidy 1879 with *blattae* Butschli 1878 as type is morphologically distinct from *Entamoeba* Schaudinn 1903 with *coli* Grassi 1879 as type They presented cogent arguments to the Commission requesting a rehearing On December 17 1954 (Opinion 312) the Commission validated *Entamoeba* as a generic name with *coli* a type species and designated *Entamoeba histolytica* as the correct name for the dysenteric ameba of man Additional generic names bestowed on the species *histolytica* such as *Poneramoeba Loschia Viereckia Caudamoeba* and *Karyamoebina* are synonyms of *Entamoeba*

IV Geographic Distribution

On first thought it may not seem relevant to the subject of this review to consider the geographic distribution of *Entamoeba histolytica* yet it has more than passing significance Originally this ameba was regarded as essentially a tropical parasite frequently with fatal outcome to the patient who harbored it This in spite of the fact that the first and several other earlier reports were from indigenous infections in eastern European countries and northeastern United States As a parasite of the Tropics it was referred to as the dysentery ameba or the agent of amebic liver abscess and as late as the first edition of Standard Nomenclature of Diseases and Operations published in the United States in the early 1930 s amebic dysentery and amebic liver abscess were the only categories available for designating *E histolytica* infection in American hospital records During the past quarter century it has been increasingly evident that *E histolytica* has a cosmopolitan distribution from the Arctic to the Antarctic in both Hemispheres and that all ages and both sexes of all races are equally susceptible to infection when given similar exposure Earlier reports of epidemic outbreaks or hyperendemicity of clinical amebiasis came most frequently from tropical areas such as Egypt (Koch 1881 Kartulis 1886 Kruse and Pasquale 1894) India (Rogers 1903) the Philippines (Strong 1901 Musgrave and Clegg 1905) and the Panama Canal Zone (James 1914 1928 Deeks 1914 Clark 1924) but as diagnostic and epidemiologic studies were increasingly pursued throughout the world high incidence of the infection was demonstrated in many temperate climates in which from time to time epidemic outbreaks

of clinical amebiasis has occurred viz Chicago (McCoy *et al* 1936 Hardy 1935) Tokyo (Ritchie and Davis 1948) South Bend *Indiana* (Brooke *et al* 1955) and England (Morton *et al* 1952) or high endemicity of clinical amebiasis prevails (Elsdon Dew 1949) on the other hand high incidence of asymptomatic amebiasis has been found in a native population in a tropical country (Faust 1958 Faust and Read 1959) Incidence figures of *E histolytica* in various countries have been assembled in recent years and are readily accessible (Anderson *et al* 1953 pp 15 27 Faust and Russell 1957 pp 195 201)

In summary it may be stated that infection with *E histolytica* has a world wide distribution is frequently more prevalent in the Tropics than in temperate or cool climates but under conditions of comparably poor hygiene and environmental sanitation it may be as serious in its clinical effects in cool as it is in warm climates

V Diagnosis of *E histolytica*

The only present day dependable criterion for the diagnosis of *E histolytica* is the microscopic demonstration of this organism and its differentiation from other microorganisms artifacts or cellular debris in the examining medium It is essential that the material for examination should be fresh and uncontaminated or if laboratory examination of the fresh specimen is not feasible it should be fixed in a medium that will preserve its cytologic properties especially those of the nucleus in a practically unaltered condition (Brooke and Goldman 1949 Sapero and Lawless 1953) Months of intensive training and experience are required to develop diagnostic competence Skilled American workers are far from agreement on the specific diagnostic criteria of *E histolytica* except for two stages viz (1) cysts larger than 10 μ containing one to four typical nuclei and rod shaped chromatoidal bodies with smooth rounded ends (using combined saline and iodine or permanent stained smears) and (2) living trophozoites that contain red blood cells extrude hyaline pseudopodia and exhibit progressive locomotion viewed in saline smears (Brooke *et al* 1953)

Yet infection with *E histolytica* is frequently not detected in direct fecal films (physiologic saline and iodine stained or hematoxylin stained preparations) Often cysts are obtained only by concentration techniques such as zinc sulfate flotation (Faust *et al* 1938) or centrifugation (Ritchie 1948) but trophozoites do not usually survive these procedures In some cases examination of repeated fecal specimens is necessary to obtain evidence of *E histolytica* infection in a positive individual (Svensson and Linders 1934 Sawitz and Faust 1942) Supplementary methods such

as saline purgation or enemas and proctoscopic aspirates will at times provide positive evidence when the examination of stools is consistently negative Moreover in a considerable percentage of cases the examiner is confronted with trophozoites or cysts of *E histolytica* which are not of diagnostic quality the trophozoites may fail to exhibit characteristic movement and the cysts may be so filled with glycogen that their nuclear structure is obscured in which cases large sized cysts may be confused with those of *E coli* and small sized cysts with those of *Endolimax nana* Thus although dependable diagnosis of this organism may readily be made when the trophozoites or cysts are typical there are many occasions when competent workers are in doubt as to the species diagnosis

Although Dobell and Jepps (1918) believed they could distinguish as many as five different races of *E histolytica* on the basis of size measurement of the cysts more recent investigations have clearly defined two distinct races those with a mean diameter of 10 μ or less for unshrunken cysts and those with a mean diameter in excess of 10 μ (Sapero *et al* 1942 Burrows 1957 Faust 1958) The interpretation of these size differences will be considered subsequently in this review

Complement fixation tests for the diagnosis of *E histolytica* were first devised by Craig (1928 1929) who utilized an alcoholic extract of antigen obtained from dysenteric stools teeming with myriads of active *E histolytica* trophozoites Later workers employing physiologic saline extracts of the ameba have failed to obtain high diagnostic pecificity with this test compared with discovery of the organism in the stools of infected patients Refined techniques including culture of the ameba with a mono bacterial associate (Bozicevich *et al* 1946) and use of pooled antigen (Bozicevich 1950) have partly solved the difficulties but the method remains unsatisfactory for clinical laboratory diagnosis Similarly the precipitin test developed by Moan (1957) has given equivocal results One probable explanation for negative immunologic tests in infected persons is that the amebae in relatively superficial mucosal lesions in the colon fail to stimulate sufficient antibody production to be detected in the serum On the other hand masses of amebae vigorously growing and multiplying in the deeper tissues are likely to produce a high level of antibody In the second place false positive reactions may result from bacterial antigen extracted from ameba cultures containing bacterial associates The most promising immunologic test of recent years is that developed by Goldman (1954) who exposed *E histolytica* and *E coli* to anti *E histolytica* fluorescein tagged conjugant and obtained specific staining reaction only for the former ameba

Some clinical laboratory workers employ culture techniques as a

supplement to fecal examination or saline purged specimens for diagnostic purposes However trophozoites of *Entamoeba coli* at times exhibit as great pseudopodial activity as do those of *E histolytica* and given the opportunity will readily ingest red blood corpuscles so that a misdiagnosis may be made unless careful attention is given to differences in nuclear structure The use of cultures to provide material for experimental studies including biochemical tests will be considered in the following section

VI Biological and Biochemical Studies

The development of *in vitro* methods for the cultivation of *Entamoeba histolytica* (Boeck and Drbohlav 1925 Dobell and Laidlaw 1926 and others) ushered in an entirely new phase of investigation on the intrinsic and extrinsic factors governing the life of this organism Rees (1955) has listed the significant accomplishments obtained from the earlier relatively crude cultures of this ameba having multiple usually unknown enterobacterial associates These include elucidation of the complete life cycle of *Entamoeba histolytica* (Dobell 1928) testing the survival of cysts deposited in water (Jones and Newton 1950) in moist soil (Beaver and Deschamps 1949a) on vegetables (Jones 1952) and in the bodies of insect vectors (Pipkin 1949) likewise the chemical sterilization of water (Stone 1937 Chang and Fair 1941 Brady *et al* 1943 Kessel *et al* 1944) and vegetables (Beaver and Deschamps 1949b) Thickness of the cyst wall has been found to effect survival under conditions of desiccation (Reardon *et al* 1952)

Refinements in culture techniques have developed along two main lines i e control of the associated bacteria and determination of the essential ingredients optimal pH and O tension for survival and growth of *E histolytica* The amebae were dissociated from their bacteria by microisolation of cysts (Rees *et al* 1950) or by use of antibiotics (Faust *et al* 1948) then reassociated with a single known bacterium (Rees and Reardon 1945 Rees *et al* 1953) suitable for abundant growth To be certain that only one pure line of the ameba was being employed clones were selected by microisolation (Rees 1942) Shaffer and Frye (1948) and Shaffer *et al* (1949) developed a clear thioglycolate culture medium preconditioned with a streptobacillus without demonstrable bacterial multiplication whereas Phillips (1950 Phillips and Bartgis 1954) substituted *Trypanosoma cruzi* for the bacterial associate The monbacterial associates commonly present in the intestinal flora of man found to be most useful in *E histolytica* cultures are *Escherichia coli* *Aerobacter aerogenes* and *Clostridium perfringens* (Rees *et al* 1953) and the unidentified nonintestinal bacterium designated as organism *t* has proved most valuable

in the development of cultures of *Entamoeba histolytica* (Rees and Reardon 1945) Some strains of *E histolytica* grow much better in monophasic than in diphasic media with coagulated slanted base Moreover in the Shaffer Ryden Frye (1949) cultures the growth of strains of *E histolytica* has been found to follow a different pattern from that exhibited in the more conventional media (Faust *et al* 1950)

Many attempts have been made to cultivate *E histolytica* axenically but all efforts have met with equivocal success at most permitting excystation of cysts in microtubes (Rees *et al* 1950) minimum growth or only survival in chick embryonic fluid (Sadun *et al* 1952) in minced chick embryonic tissue (Shaffer *et al* 1953) or in HeLa tumor cells (unpublished data of Dr G M Carrera) These trials suggest that *E histolytica* lacks certain essential enzymes for its metabolism which are provided by certain bacterial associates and *Trypanosoma cruzi*

Extensive and intensive study has been made of the optimal ingredients for *E histolytica* in the culture medium With relatively few exceptions rice flour has been incorporated in the medium (Faust and Read 1959) owing to its ready availability and the small size of the rice starch grains Hilker *et al* (1957) confirmed the findings of Hallman and DeLamater (1953) and Baerstein *et al* (1954) that *E histolytica* produces amylase and additionally demonstrated maltase activity However for large race strains of *E histolytica* larger sized starch grains such as those of plantain yucca Irish potato white sweet potato and wheat are readily utilized since these starches contain large amounts of amylopectin (branched chain component) which is more easily hydrolyzed by the ameba than amylose (Faust and Read 1959) Nevertheless it is possible that one difficulty in getting small race strains of *E histolytica* to develop in cultures with rice flour results from the fact that even the small rice starch grains are at times too large for these trophozoites to ingest (Faust and Read 1959)

In monoxenic cultures with *Escherichia coli* and organism *t* Rees *et al* (1944 1953) found that starch cholesterol and probably vitamin B complex are essential for growth of the ameba (Vitamin B is produced by *Escherichia coli* but not by organism *t*) Jacobs (1950) concluded that only traces of factors supplied by the bacterial associate are necessary for luxuriant growth of the ameba Hansen and Anderson (1948) devised an essentially synthetic culture medium in which *Entamoeba histolytica* and a single bacterial associate were carried through many subcultures Twelve amino acids were included as well as approximately isotonic concentrations of buffering salts which maintained a favorable pH of 6 1–7 9 in spite of changes produced by the bacteria

Another important aspect of the growth requirements for *E histolytica* is anaerobiasis (Snyder and Meleney 1943 Chang 1946 Balamuth and Howard 1946) The last two groups of investigators discovered that pre conditioning the medium with the suitable bacterium adjusts the oxidation reduction potential to a low level and provides certain essential enzymes allowing the amebae to grow and multiply

Encystation in culture as in the appropriate host animal is another aspect that has been studied by several workers Snyder and Meleney (1943) discovered that the factors inducing encystation *in vitro* are simpler than those governing vegetative propagation Although Cleveland and Sanders (1930) Chinn *et al* (1942) and Chang (1942 1946) were of the opinion that certain bacterial associates favor encystation and Chang (1946) concluded that a pH of 6 8 to 7 0 and low oxidation reduction potential were the important conditioners Everritt (1950) concluded that density of population was probably the most significant factor Following moderate growth in a starch free monophasic medium trophozoites of two strains of *E histolytica* were transferred to the same preconditioned environment containing starch Encystation was directly proportional to the logarithmic increase in the size of the population which in turn was proportional to the horizonal surface available to the amebas Encystation occurred gradually throughout a 12 to 16 hour period and was disturbed by agita tion of the medium Everritt (1950) noted different population levels in two different strains and the writer of this review has more recently found similar differences in the cultivation of fifteen different strains (twelve of the large race and three of the small race)

Biochemical studies on *E histolytica* trophozoites have provided a num ber of interesting observations These may be divided into the intrinsic properties of the ameba and those related to its host parasite relationship

Proteolysis Craig (1927) obtained an alcoholic heat labile extract from cultured *E histolytica* trophozoites which was hemolytic for red blood cells and cytolytic for intestinal epithelium Alcoholic extraction of as sociated bacteria and saline extraction of *E histolytica* failed to demon strate these activities Hallman *et al* (1950) Rees *et al* (1953) and Balamuth and Brent (1954) demonstrated gelatinase activity in strains of this organism which Balamuth (Balamuth and Thompson, 1955) con sidered to be an extracellular protease activity of free living as well as parasitic amebae Rees *et al* (1953) provided evidence that this activity of the ameba is responsible for liberating starch grains from rice flour agglomerates thus enabling the ameba to ingest and then digest the indi vidual grains Shaffer (1953) found that trypticase was the only one of

seven peptones tested in the thioglycolate culture medium which supported growth of *E histolytica*

Glycolysis A considerable volume of investigation on cultured *E histolytica* has provided convincing evidence of the need for starch in the metabolism of this organism (Balamuth and Thompson 1955) Except for special studies rice starch with associated proteins and lipids in rice flour has become the routine carbohydrate ingredient in cultures However early in the *in vitro* cultivation of *E histolytica* St John (1930) utilized whole wheat flour and Dobell and Laidlaw (1926) tested starches of rye buckwheat and maize More recently Benham and Havens (1958) found starch grains of *Amaranthus paniculatus Kochia scoparia* and *Mirabilis jalapa* which have particle sizes of 2 μ 0 5–1 0 μ and 1–2 μ respectively to be satisfactory substitutes likewise Faust and Read (1959) tested the capacity of large race *E histolytica* to utilize starches having much larger sized grains

Hallman *et al* (1950) were able to obtain growth of *E histolytica* in an essentially synthetic culture medium containing soluble carbohydrates viz glucose and glycogen or maltose However soluble carbohydrates provide a more available food for the associated bacteria which with the exception of *Clostridium perfringens* do not primarily utilize starch in their metabolism It thus appears that a starch satisfactory for the ameba is more conducive to amebic growth without overgrowth of the bacteria (as measured by CO production) than is a soluble carbohydrate Nevertheless the substitution of sugars for starches in special culture media e g Shaffer Frye and *E histolytica-T cruzi* association has provided exceptions to this general conclusion

Hilker *et al* (1957) in a study of the carbohydrate hydrolyzing enzymes of this ameba demonstrated the presence of both amylase and maltase and concluded that particle size apparently is not the only factor involved Hilker (personal communication 1958) believes that the degree of utilization of starch (e g the splitting of ingested starch grains and converting them into sugars) depends on whether the starches contain primarily the easily hydrolyzed branched chain amylopectin or the more resistant straight chain amylose

Utilizing ameba streptobacillus and ameba *T cruzi* association in cultures Entner and Anderson (1954) concluded that the slight activity in degradation of glucose or maltose to succinate and lactate by resting suspensions of *E histolytica* resulted from endogenous metabolism Becker and Geiman (1954) employing glucose C^{14} labeled substrates of antibiotic inhibited multibacterial associates with *E histolytica* under anaerobic conditions demonstrated from cultures and lysates that C^{14} became incor

porated as glycogen and CO_2 but was not detected in bacterial controls Significant biochemical studies on the carbohydrate metabolism of *E histolytica* have been conducted by Kun and Bradin (1953) and Kun *et al* (1955 1956) Under anaerobic conditions suspensions of intact and macerated trophozoites of several cultured strains produced appreciable CO_2 and H_2S from glucose fructose or mannose and cysteine as sub strates to a lesser degree when sucrose or galactose was substituted for the other sugars The associated bacteria contributed negligibly if at all to the production of the end products and there was no essential quantita tive difference in the reaction of the several ameba strains tested These experiments suggested that *E histolytica* contains a product of oxidation of a triose phosphate the oxidation of which is measured by the rate of H_2S production The evolution of CO_2 and H_2S roughly paralleled the number of amebae present in the test CO_2 production resulted from de carboxylization of pyruvate the product of glucose fermentation On the basis of various inhibitors in the substrate the metabolic pathway of catabolism was found to be different from that of the Krebs system with lactic acid production but indicated a coupling between dehydrogenase activity and sulfur reducing enzyme systems The work of Seaman (1953) on the recovery of succinic dehydrogenase from *E histolytica* free cells supports this view

More recently Hilker and White (1958 1959) have demonstrated that in addition to employing the more conventional glycolytic pathway for the utilization of glucose *E histolytica* elaborates enzymes for an alterna tive pathway previously known to be employed only by certain polar flagellated bacteria This work has been confirmed by Entner (1958) who grew *E histolytica* in the Shaffer Frye medium then supplied whole washed amebae with radioactive glucose *1* C^{14} He found labeling pre dominantly in the methyl rather than the carboxyl group and absence of CO_2 labeling indicating failure to employ the pentose phosphate pathway The associated streptobacilli when tested similarly by Entner failed to demonstrate enzymes capable of utilizing the alternative pathway

Lipolysis Cholesterol is an essential component of growth producing substances for *E histolytica* (Snyder and Meleney 1943 Rees *et al* 1944) Moreover Griffin and McCarten (1949) developed more luxuriant amebic cultures when a very small amount of oleic acid (20 mg/ml) was added to cholesterol in the culture medium although they found that 60 mg/ml was toxic and prevented survival of the amebae

Vitamin requirements In earlier studies with a monobacterial associate Rees *et al* (1944) prepared a variety of culture media in Florence flasks incorporating various vitamins Highest average ameba production was

obtained with whole egg second best average yields with eight members of the vitamin B complex and riboflavin plus cholesterol The vitamin B components were regarded as essential to optimal growth although they were ineffective without cholesterol Later studies by Rees and associates (1953) supported this earlier conclusion Experiments by Hallman *et al* (1950) in defined synthetic media confirmed this line of investigation

In summary the *in vitro* cultivation of *Entamoeba histolytica* opened a broad area of study concerning both intrinsic properties and extrinsic requirements for the survival and growth of this ameba and biochemical studies provided data concerning the metabolic needs of this organism its metabolic pathways in the utilization of carbohydrates amino acids and proteins lipids and vitamins Only a beginning has been made in this field which will challenge investigators for many years

VII The Relationship of *E histolytica* to Its Host

In addition to the three species of *Entamoeba* that commonly parasitize man (*E histolytica E coli* and *E gingivalis*) twenty four other described species of this genus are listed by Kudo (1954) These include two each from the horse and pig one each from cattle sheep rabbit guinea pig rat and mouse ground squirrel domestic fowls three from turtles one from snakes one from frogs and several from invertebrates Wenyon (1926 pp 225–235) included in the genus *Entamoeba* twenty three species described from hosts other than man Eight of these are not in Kudo s list and seven species in Kudo s list are not mentioned by Wenyon Even though some of the species inadequately described may belong to other genera of amebae and some of the names probably are synonyms it is clear that species of *Entamoeba* have become adapted to a wide range of hosts almost invariably at some level of the digestive tract Moreover *E moshkovskii* has been described from four widely separated areas as a free living organism in sewage overflow and stagnant water Of the valid species of *Entamoeba* only *E histolytica E invadens* from reptiles (including turtles) and possibly *E ranarum* from frogs have been demonstrated to have the capacity to invade the intestinal wall and produce disease Insofar as present information indicates all the other parasitic species of this genus live as commensals in their respective homothermic or poikilothermic hosts *in equilibrium* with the animals that support them

In a general consideration of host parasite relationship Audy (1958) has stated that a particular potential pathogen presented to all possible hosts and host species will fail to infect most or many of them while it will infect others and cause either no apparent derangement (inapparent

infection) or disease (apparent infection) Each pathogen will on testing provide us with an array of host species through which the parasite has passed in its evolutionary adaptations These remarks are peculiarly applicable to the species of *Entamoeba* and are especially relevant to *Entamoeba invadens* and *E histolytica* *E invadens* is best known as a parasite of snakes in which it produces fulminating infection at times causing epizootics which indiscriminately kill a large percentage of various species in confined quarters as in a zoological garden (Geiman and Ratcliffe 1936 Ratcliffe and Geiman 1938) Yet in turtles (Meerovitch 1957) and in domestic lizards this ameba is a harmless intestinal inhabitant This aspect of host species parasitism will be considered later under the subject of nutritional factors in relationship to pathogenicity

Infection with *E histolytica* has been studied rather intensively in a variety of naturally and experimentally infected hosts including man Natural infection with an ameba indistinguishable morphologically and in its life history from *E histolytica* has been found in Old World and New World monkeys (Eichhorn and Gallagher 1916 Dobell 1919 1928 Kessel 1927 Hegner and Chu 1930 Ratcliffe 1931 Knowles and Das Gupta 1934 Johnson 1941) in dogs (Kartulis 1904 Darling 1915 Fischer 1918 Bauche and Motais 1920, Boyd 1931 Faust 1931) in rats (Lynch 1915 Chiang 1925 Andrews and White 1936) and in pigs (Kessel 1928b) Experimentally all these animals as well as rabbits (Tobie 1949 Hunninen and Boone 1957) guinea pigs (Carrera and Faust 1949 Taylor *et al* 1950) hamsters (Reinertson and Thompson 1951) and kittens (Cleveland and Sanders 1930 Meleney and Frye 1932) have been utilized in studying the life cycle of *E histolytica* in physiopathologic investigations and in testing new chemotherapeutics

The characteristic method by which exposure to *E histolytica* occurs in nature is the ingestion of the encysted stage of the organism although under natural conditions (Boyd 1931) and experimentally (Swartzwelder 1939) dogs have become infected from oral introduction of fresh trophozoites into the digestive tract The rapidity with which the cysts are emptied into the small intestine excyst there and the metacystic trophozoites pass into the cecal area depends in part on the species of host its digestive enzymes the length of its small intestine and whether it contains an appreciable amount of food in process of digestion (Hegner *et al* 1932 Swartzwelder 1939 Tsuchiya 1939) Excystation typically occurs in the lower portion of the ileum but may be delayed until the cysts reach the cecum and at times viable mature cysts may possibly be evacuated in the feces of the exposed host without excystation (Faust 1941) Unlike the observations of Dobell (1928) on excystation of *E histolytica*

in vitro Hegner *et al* (1932) and Swartzwelder (1939) in *in vivo* studies found no more than four metacystic trophozoites derived from each mature cyst

Colonization of *E histolytica* in the susceptible host depends on a number of interchangeable factors including the viability of the cysts ingested the number of metacystic trophozoites that arrive in the large intestine as well as opportunity for them to make contact with the intestinal wall and to become temporarily lodged in the crypts or apposed to the mucosa Neal (1958) has stated that bacteria are required for the amoebae to manifest their pathogenic properties but the presence of virulent bacteria which injure the mucosa is not necessary The [illegible] writer would prefer to modify this statement by substituting the [illegible] to survive and colonize for to manifest their pathogenic properties [illegible] In other words although *E histolytica* is provided with enzymes [illegible] ingested starches e g amylase and maltase (Hilker *et al* 19 7) [illegible] tissue cells and connective tissue e g hyaluronidase (Bradin, 1[illegible] [illegible] elaborates acid phosphatase (Carrera and Changus 1948) this [illegible] lacks certain essential enzymes which are needed for it to survive [illegible] or in the intestinal lumen However once it has entered the [illegible] mucosa and has penetrated below the surface layer or has been [illegible] by the circulation to the liver and other secondary sites bacteria [illegible] needed for its propagation within the tissues (Cleveland and Sanders [illegible] Carrera and Faust 1949 Rees *et al* 1954 Reinertson and T[illegible] 1951) The secondary sites are characteristically bacteriologically [illegible]

In the dog as in man intestinal amebiasis may be [illegible] evidence of relatively rapid repair of eroded mucosa (Harris 1[illegible] 1932 1941 Faust and Kagy 1934) may be moderately [illegible] portunity for entry into the mesenteric veins and for est[illegible] secondary centers of activity in the liver and other viscera ([illegible] and Lafleur 1891 James 1928) or it may be extensive [illegible] times result in perforation (Musgrave 1910 Clark 192[illegible] Faust and Kagy 1934 Elsdon Dew 1949) In monkeys [illegible] usually relatively superficial but occasionally deep penetration [illegible] (Johnson 1941 Bond *et al* 1946) In kittens guinea [illegible] the ulceration is typically extensive and deep with fatal termination [illegible] 1929 Meleney and Frye 1934 Carrera and Faust 19[illegible] In rats because of the paper thin character of the [illegible] lesions are relatively superficial and are characteristically [illegible] 1946 Neal 1957) One may possibly conclude that the [illegible] satisfactory experimental animal for studying acute [illegible] though the rabbit and guinea pig have served well [illegible]

pathogenic capacity of different strains of *E histolytica* (Tobie 1949 Krupp and Faust 1959 Hunninen and Boone 1957) and the hamster has been useful in providing information on initial hepatic colonization of this organism (Reinertson and Thompson 1951 Rees *et al* 1954) The dog especially the puppy has served as a valuable test animal providing data on lesions varying from those essentially inapparent to those of a fulminating type (Faust and Kagy 1934 Tobie 1940) Probably the monkey is not a particularly useful experimental host except in screening potential antiamebic drugs (Anderson and Koch 1931 Anderson and Anderson 1950) The rat was first employed routinely by Jones (1946) in chemotherapeutic studies and more recently has been studied by Neal (1957) for indices of pathogenicity of different strains The advantage of using the laboratory rat is that it is from pure inbred stock and its diet is standardized the disadvantage of its use is that the amebic lesions in its intestine are typically superficial and do not represent the protean varieties of deep ulceration characteristic of many cases of human amebic colitis

Information which has thus far been presented with reference to host infection with *E histolytica* has been confined to host species populations and has not taken into consideration the many supervening factors in different individuals of a species that play important roles in infection with this organism such as susceptibility diet environmental influences difference in pathogenic capacity of different strains of *E histolytica* and exposure dosages to which the individual is subjected Each of these topics will now be considered briefly

Individual susceptibility Audy (1958) has remarked that two individuals may differ markedly from each other in their response so that it is important to distinguish individual from species susceptibility Given equal degree of exposure to cysts of *E histolytica* all or a majority of exposed individuals human or experimental may become infected or only a small proportion may become parasitized The reasons for this difference are not entirely clear but such difference has been abundantly demonstrated statistically In discussing susceptibility to viruses Beard (1958) refers to the possible influence of genetic constitution hormonal balance age and many other factors that strongly influence or condition the degree of individual susceptibility

Diet It has become increasingly evident that the nutritional state of the host has an important bearing on susceptibility to infection with *E histolytica* or at least to the consequences of the infection Elsdon Dew (1949) and Elsdon Dew and Freedman (1952) studied this problem in South Africa where amebiasis behaves quite differently in three racial

groups viz with acute fulminating infection in the native Bantu living primarily on a maize diet with an essentially carrier condition in the Indian population subsisting on a basic curry and rice diet with vegetable supplements and with relatively infrequent infection of clinical grade in the white population having a well balanced diet with adequate proteins and vitamins

Recent studies by Faust and Read (1959) have been concerned with the problem of diet in respect to clinical manifestations in a economically poor population of Cali Colombia Although the average incidence of *E histolytica* primarily large race strains is 40% in this population in a period of more than three years only three cases of mild clinical amebiasis have been encountered and physical examination including proctoscopy in a representative sampling has failed to reveal amebic ulceration or abdominal tenderness The infected individuals are almost exclusively cyst passers they lose their infection over a period of months and with equal frequency become reinfected Their formed or semiformed feces contain an appreciable amount of undigested starch grains identified as those of plantain and yucca which constitute the basic food of this population Proteins vitamins and essential minerals are consumed in negligible amounts Under such a dietary imbalance the low caloric starch intake is not well utilized owing to inadequate pancreatic and intestinal enzyme production resulting in the passage of undigested starch grains through the large intestine Culture studies have demonstrated the capacity of this ameba to ingest these starches essentially as readily as rice starch It is predicated that this supply of readily available starches in the lumen of the large intestine may explain the apparently superficial relationship of the ameba to the intestinal wall in this population so that the clinically inapparent infection constitutes an essentially commensal relationship It is possible that this study on relationship of diet to amebiasis may provide a partial explanation for differences in manifestation of *E histolytica* infection in the different racial populations in Natal South Africa and elsewhere

The observations referred to above on the availability of particulate carbohydrates for *E histolytica* in its immediate environment in the large intestine of the human host parallel those of Meerovitch (1957) concerning the biology of *E invadens* He considers encystation as a necessary stage in the life history of this and other species of parasitic amebae One of the essentials for abundant encystation is a supply of starch in the immediate environment *With this condition fulfilled there is no necessity for tissue* invasion

Earlier studies along this same line were conducted by Lincicome

(1942) using rhesus monkeys On a high carbohydrate low protein diet cysts of *E histolytica* appeared daily in the stools with maximum cyst production at periods of approximately every 7 days With increased protein intake cyst elimination rapidly declined and then disappeared

Environmental influences Epidemic outbreaks of severe amebiasis in earlier decades in Calcutta Cairo Manila the Panama Canal Zone and elsewhere as well as more recently in Chicago South Bend (Indiana) Tokyo and in an air force base in England resulted from drinking water grossly contaminated with sewage containing viable cysts of *E histolytica* Basically in all these areas there was already a high endemicity of amebiasis in the native populations Correction of the sanitary difficulties prevented further epidemic outbreaks Likewise in cities like New Orleans improvement in general sanitation has greatly lowered the incidence of endemic amebiasis during the last quarter century e g from 10–16% to less than 2% Nevertheless in many tropical regions as well as in children s homes mental hospitals and prisons in cooler climates lack of potable water due to inadequate sewage disposal coupled with poor environmental sanitation and personal hygiene have been responsible for maintaining a high level of endemic amebiasis with periodic epidemics

VIII Pathogenicity of Different Strains of *E histolytica*

Many studies have been conducted to test the pathogenic capacity of different strains of *E histolytica* in temperate areas and to a lesser extent in the Tropics It is today generally agreed that different strains of this organism have different pathogenic potentials at least under the environmental situations in which they have been studied Within a limited period of observation and experimental testing in the kitten dog or rat strains of low pathogenic capacity do not develop greater potential except at times by rapid repeated serial passage from one animal to another (Meleney and Frye 1933 1935 Faust and Swartzwelder 1935 Faust *et al* 1946) Meleney and Frye (1937) obtained the highest pathogenic index in their investigations from strains from the Chicago epidemic of 1933 Yet neither Frye and Meleney (1933) nor Faust and Swartzweder (1935) were able to demonstrate that high pathogenicity resulted from the presence or absence of associated pathogenic bacteria Neal (1958) states that virulent bacteria which injure the mucosa are not necessary for the ameba to manifest its pathogenic properties Nevertheless as indicated earlier it is essential for *E histolytica* trophozoites to have an appropriate bacterial associate or associates in the intestinal lumen prior to their entrance into the tissues Phillips *et al* (1955) put this thesis to a critical test by comparing the results obtained following intracecal inocu

lation of a culture of *E histolytica* with *Trypanosoma cruzi* as the monoxenous associate into 35 germ free and 37 other guinea pigs having conventional intestinal fauna and flora None of the former group developed lesions and none developed colonies whereas 34 of the latter developed acute amebic colitis and the remaining 3 demonstrated colonization at the time of sacrifice on the twenty first day It was concluded that associated intestinal bacteria such as *Escherichia coli* and *Aerobacter aerogenes* participate in the development of amebic infection even though *E histolytica* is unquestionably the causative organism of intestinal amebiasis

Another aspect of strain pathogenicity is whether a once potent strain of *E histolytica* will lose its virulence after prolonged cultivation Investigators have differed widely on this point although there is unanimity with reference to the maintenance of virulence in successive animal passages Chang (1945) and Neal (1956) demonstrated decreased infectivity and pathogenicity after prolonged *in vitro* cultivation but restoration of these characteristics after direct animal transfers Faust *et al* (1948) studied fourteen strains and concluded that culturability infectivity and pathogenicity were not necessarily correlated and that these properties tended to vary in different strains De Carneri (1958) was able to step up the pathogenic index of three strains (one from a case of chronic amebic colitis one from a culture obtained from a brain abscess and one from a cyst passer) by first introducting them into the liver of the golden hamster He concluded that *in vitro* cultivation for three to twelve years with temporary loss of virulence does not deprive the strains of their intrinsic pathogenicity The observations of Krupp and Faust (1959) have a direct *in vivo* bearing on this point Material for guinea pig inoculations consisted of fourteen cultured strains obtained from symptomless human carriers in which there was substantial evidence that the amebae were living essentially as commensals in the lumen of their hosts Yet more than 50% of 103 test animals inoculated with trophozoites intracecally exhibited amebic ulceration at autopsy including some guinea pigs from every strain and in some instances all animals in the same series Possibly the high rate of encystation observed in the cultures of these strains is a confirmation of Chang s belief (1945) that encystation enhances the virulence of a strain that has temporarily lost its pathogenic potential from prolonged *in vitro* cultivation

Thus far the discussion of pathogenicity of *E histolytica* has not considered as such the views of many European investigators who regard the indigenous strains of their countries as essentially nonpathogenic whereas they classify the strains introduced from the Tropics as patho

genic Since relatively few critical tests have been conducted by these workers in susceptible animals it is difficult to compare their strains with those in North America which have been repeatedly subjected to experimental animal study The most active present day proponent of the European concept is Hoare (1949 1950 1952 1957 1958) who has stated the case clearly (1958) by dismissing the small race as an inoffensive non pathogenic ameba which he believes should be placed in the same category as *Entamoeba coli Endolimax nana* and *Iodamoeba butschlii* and dividing the large race of *E histolytica* into (1) the nonvirulent type corresponding to Brumpt s *E dispar* (Brumpt 1925) and (2) the virulent type corresponding to Brumpt s *E dysenteriae* This categorization is referred to as neo dualism Continuing with this concept Hoare (1958) believes that a high percentage of symptomless carriers harbor the avirulent type of the large race whereas other carriers are infected with the virulent type which may produce symptoms under favorable conditions Only this latter type is clinically significant yet it can be differentiated from the avirulent type solely by animal experimentation If this concept is valid a considerable amount of amebiasis in the temperate climates of North America and similar latitudes in Argentina Uruguay and Chile must be considered as having been introduced at an earlier date from tropical areas and then firmly established in the populations of European origin in these countries where clinical grade infections constitute an important subject in diagnosis and treatment On the contrary there is no substantial evidence supporting the establishment of introduced virulent strains of *E histolytica* into the United States following the return of veterans of World War II from tropical areas overseas (Lincicome *et al* 1950)

From an academic standpoint the neodualistic concept of Hoare (1958) is interesting and will be attractive to many students of medical protozoology From the standpoint of the diagnostic laboratory and the practicing physician it poses serious questions Should the competent parasitologist in the clinical laboratory differentiate the small race *E histolytica* from the large race? For the past three years the writer has done this in reports incorporated in case histories in a special family care study and in a smaller series of private patients but it is doubtful if this distinction is considered important by the attending physicians More serious is the morphologic similarity of the postulated avirulent and virulent types of the large race of this ameba Should the physician wait until the patient manifests definite symptoms of amebic colitis or amebic hepatitis before initiating antiamebic therapy or should he consider all cases of the large race as potentially dangerous and therefore following a reliable laboratory diagnosis treat all positive cases under his care irrespective of whether they have symptoms or are symptomless at the time?

IX The Mechanism of Tissue Invasion

There is general agreement that *E histolytica* trophozoites require a suitable bacterial associate to colonize in the large intestine of the appropriate host and more recent investigators (Carrera and Faust 1949 Phillips and Bartgis 1954 Neal 1957) have produced evidence that bacteria are not needed once the ameba has entered the intestinal mucosa However there is no convincing demonstration as to the exact mechanism of tissue invasion although it is most likely that this results from contact of the ameba s lytic enzymes with the mucosa (Maegraith *et al* 1959) It is obvious that a mucosa already damaged by abrasion by pathogenic bacteria or by other agents may serve the ameba in taking the initial step but many workers believe that the ameba is able to enter without such preconditioning of the epithelium Faust (1931) Faust and Kagy (1934) Meleney and Frye (1934) and others have observed lesions produced by *E histolytica* in experimental animals as early as 24 hours after inoculation of cysts by mouth or of trophozoites intracecally These were slightly elevated discrete pinpoint ulcers in an otherwise intact mucosa and in section exhibited no evidence of bacterial association Craig (1927) and subsequent students of amebiasis (Rees *et al* 1953 Deschiens 1950 Neal 1956) have demonstrated proteolytic activities of the ameba and Bradin (1953) but not DeLamater *et al* (1954) has isolated hyaluronidase from trophozoites after they have been associated with host tissues several demonstrations have been made of proteolytic enzymes elaborated by this ameba Likewise there is satisfactory evidence that *E histolytica* trophozoites are mechanically able to squeeze between mucosal cells of the large intestine (Faust and Kagy 1934 Hoare and Neal 1955) Thus the mechanism of penetration may be regarded as that of progressive lytic digestion aided by mechanical efforts The larger the ameba and the greater its activity the more rapidly is it able to proceed in its cellular destruction with almost negligible host cell reaction when its entry and progress are bacterially uncomplicated The above information is factual but it leaves much to be desired as to the biochemical activities of the ameba at the point of dissociation with the bacteria of the intestinal lumen and initiation of host cell association

X Designation of Different Races and Strains of *E histolytica* (*sensu lato*)

As pointed out in a preceding section there is considerable disagreement among parasitologists with respect to the nomenclature of the different racial groups of *E histolytica* based on size morphologic characters and differences in pathogenicity of different strains Many workers remain

unitarian in their concepts from the nomenclatural viewpoint i e that *all organisms conforming to the criteria of E histolytica (sensu lato)* should be designated as *E histolytica* even though substantial evidence has been provided that there are two distinct races based on mean size differences (Sapero *et al* 1942 Burrows 1957 Faust 1958) and that they are morphologically distinguishable (Burrows 1957 Faust 1958) Moreover many investigators are convinced that the small race in man or test animals is at times capable of tissue invasion The experiments by Kessel (1928a) and Tobie (1940) included strains of the small race obtained from human carriers these produced typical amebic lesions in the large intestine of kittens and dogs respectively Recently Krupp and Faust (1959) demonstrated tissue invasion in the guinea pig of a small race of *E histolytica obtained from an asymptomatic human case Perhaps the* views of these students of amebiasis may be stated as follows Because of its diminutive size and lesser enzyme activity production the small race less frequently has the capacity to invade tissues and when it does invade produces less destructive tissue damage In contrast the opposing group of workers considers the small race to be a distinct nonpathogenic species which they designate as *E hartmanni* (Burrows 1957 Hoare 1958) although there is considerable doubt that von Prowazek s (1912) small quadrinucleate ameba from a Samoan woman designated as *Entamoeba hartmanni* is the same ameba as that which today constitutes the small race of *E histolytica* Perhaps a tentative solution to this controversy *might be the designation of the small race as a subspecies* (the morphologic characters of the nuclei do not justify full species rank) e g *E histolytica hartmanni* as suggested by Hoare (1949) but without implication of its virulence except for its presumably low potential for producing proteolytic enzymes

Greater difficulty arises in an attempt to dispose of the controversy concerning nonpathogenicity or pathogenicity between different strains of the large race of *E histolytica* Here no size or morphologic differentiation is postulated only one of pathogenicity Hoare (1958) recognizes a single species name i e *histolytica* for both types although he regards them as distinct as did Brumpt (1925) when he named the former *E dispar* and utilized *E dysenteriae* for the latter Although degrees of pathogenicity of *E histolytica* (large race) have been *repeatedly demon*strated failure to discover definite clinical evidence in human infections or substantial lesions in experimentally inoculated laboratory rats does not per se warrant the assumption that there is a completely nonpathogenic variety of the large race *E histolytica* prevalent in Europe and perhaps in other temperate areas of the world This is just as inadvised as to state

categorically that all large race tropical strains of *E histolytica* are invariably highly pathogenic for man when evidence is beginning to indicate that this is not the case (Faust 1958) even though intrinsic capacity for their high level pathogenicity has been demonstrated in test animals (Krupp and Faust 1959) The proponents of the neodualistic doctrine (Hoare 1958) apparently fail to consider the nutritional and other factors that enter into the human host parasite relationship in this and many other parasitic infections Even from a strictly academic viewpoint the observational evidence presented is not adequately documented and therefore the neodualistic view does not warrant acceptance without much more precise scientific substantiation

XI Conclusions and Summary

Information that has been accumulated concerning *Entamoeba histolytica* since its discovery in 1875 constitutes a vast amount of data on both the clinical and laboratory aspects of this protozoon A great deal of progress was made in the earlier decades on the pathologic and clinical aspects of severe symptomatic amebiasis Later there was the discovery that the infection was not necessarily detectable by clinical methods and that reliable laboratory diagnosis of the organism in its cystic as well as vegetative stage is required Discovery of methods for *in vitro* cultivation of *E histolytica* made possible elucidation of its complete life cycle and experimental u e of several pecies of susceptible laboratory animals provided abundant opportunity to make *in vivo* investigation of the relationship of the ameba to its host as well as in the screening of potential antiamebic drugs One of the most significant findings was the requirement for a suitable bacterial associate to supplement the metabolic needs of the ameba in its initial colonization in the intestinal lumen together with evidence that this association is not required once the ameba has penetrated host tissues Pathogenicity studies of different strains in man and laboratory animals have likewise added valuable information although it should be emphasized that data obtained from tests in lower animals are not necessarily directly applicable to the human host

Differences in pathogenic capacity of different strains have convinced some workers that *E histolytica* is more than one species entity Possibly a more suitable reference would be the *Entamoeba histolytica* complex

In this review it has been possible to mention only a portion of the many stimulating studies that have been conducted with *E histolytica* as the focal point of investigation Many of the problems which have been undertaken have as yet reached only partial solutions Several of the differences in interpretation of the findings will undoubtedly be resolved as more refined

methods are employed and with equal likelihood divergent opinions will tend to be satisfactorily adjusted when their proponents understand not only the intrinsic variations in this organism (with possible genetic implications) as seen under different conditions but also differences in host infectibility and reactions under variable conditions of climate diet physiological host state intercurrent diseases and opportunities for exposure to infection with *E histolytica*

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Patrick Manson as a Parasitologist

A CRITICAL REVIEW

SIR PHILIP MANSON BAHR

I Introduction

It has been my good fortune to have access to the Diary which Patrick Manson (who may well be regarded as the father of tropical medicine) wrote for thirty five years In addition I have been able to consult family papers letters and transcripts of others preserved in his records It is in fact an account of all the investigations he undertook in his early years References are made also to the Journal which he kept in Formosa from 1866 to 1870 and to the notes on the literature of filariasis which he composed in the British Museum in 1875

It will be recalled that in 1935 I wrote a description of this Diary The Bible of Tropical Medicine and gave some excerpts from its pages (1935 *Trans Roy Soc Trop Med Hyg* 29 (1) 179–190)

II Preliminary History

Patrick Manson was born on October 3 1844 the second son of a family of nine in the village of Old Meldrum some 16 miles north of Aberdeen. He was a good Scot and came from a family long established in the district His father John Manson was laird of Fingask and also Manager of the British Linen Bank His mother a woman of great character was a distant relative of David Livingstone



FIG 1 The Manse Old Meldrum Aberdeenshire Scotland where Manson was educated It was on the roof of this house that he shot the cat with a tapeworm that gave him his introduction to helminthology

Young Patrick was educated at the gymnasium of Aberdeen and subsequently at the University of that city He was first designated as an engineer at the iron foundry of his mother s family but the idea was abandoned as he suffered from a spinal curvature from which he subsequently *developed a paretic affection and tremor of the right hand* which remained with him for life He had already shown an interest in helminthology when as a schoolboy of 14 he shot a cat on the roof of the local Manse (Fig 1) and studied a tapeworm which he extracted from it intestines *After qualification at the age of 21 he became* pathologist to a mental asylum in Durham and then emigrated to Takao (Takow) in southern Formosa (1866) where he remained till 1870 He then moved to

Amoy in southern China on the Bay of Hai Tau and there he laid the foundations of the new science of tropical medicine

In 1875 Manson returned to Scotland he was married in December of that year During his stay in Great Britain he made strenuous efforts to learn all he could about tropical diseases—without avail till eventually he *found in the library of the British Museum the writings of one Timothy* Lewis of Calcutta on filariasis these he transcribed into a notebook that has been preserved It is recorded that before he set sail for China he had constructed a working hypothesis on the location of the adult parasite in the body

In 1876 he returned to Amoy but this time the possessor of a compound microscope Very soon after his return he was able to confirm Lewis discovery of the microfilariae in the blood to establish the phenomenon of nocturnal periodicity and finally to work out the development of *Filaria bancrofti* in *Culex fatigans* this fundamental discovery he published in *1878*

The results were presented to the Linnean Society of London through its President Spencer Cobbold It was at this meeting that critics interposed and heckled Cobbold with questions such as this Did Manson furnish the embryo filariae with watches so that they should know what time to get up and what time to go to bed? And an old man with a skull cap and nearly 90 years old visited the School of Tropical Medicine in London in 1939 and related as sole survivor of that famous occasion how one member had exclaimed that what they had just heard was either the work of a genius or more probably the ravings of a drunken Scotch doctor in far-off China where as was well known they all drank whisky to excess! *However nothing daunted* Manson repeated and amplified this work in 1883 communicating the esults as before through Spencer Cobbold to the Linnean Society in 1884 In Amoy he did a great deal of other important work including the discovery of *Distoma ringeri Ligula mansoni* various avian filariae and *Oxyspirura mansoni* in the eye of domestic hens He also paid attention to skin fungi discovered *Tinea imbricata* and was the first to employ incubating hens eggs as a medium in an attempt to cultivate leprosy bacilli

In December 1883 he moved to Hong Kong where he practiced till 1889 There he described sprue in 1886 cured Li Hung Chang the great man of China of a sublingual abscess which had been mistaken for carcinoma and made a great reputation for himself as a successful practitioner He founded the Medical Society of Hong Kong and also the Medical School which subsequently became the University of that Colony He also became interested in malaria and undertook what he called Fool Ex

periments He returned to Scotland in 1889 intending to retire but in the following year he moved to London where he continued to work till 1913

This was his most fruitful period during which he made more discoveries in filariasis worked out the life history of the Guinea worm (*Dracunculus medinensis*) and most important of all made valuable contributions to the life history of the malaria parasite In 1894 he formulated the mosquito malaria hypothesis He became consultant in 1897 to the Colonial Office where he was closely associated with Mr Joseph Chamberlain This stimulated his ambition to found a School of Tropical Medicine which came to fruition in October 1899 He continued to direct and teach in this school almost to his death on April 9, 1922 This probably was his greatest contribution to tropical medicine and therefore he is venerated as the father of this subject Contemplation of all that this great man achieved durin his active career should take into account the fact that he was a lifelon sufferer from gout which greatly hampered his activities

Filariasis Due to Wuchereria bancrofti Summary of the Most Important Disco eries

M Demarquay 1863 first described microfilariae in hydrocele fluid

O Wucherer 1866 found microfilariae in chyluria and hematuria in Brazil

T R Lewis 1872 found and described microfilariae in blood and urine of Hindoos in Calcutta

P Sonsino 1874 described microfilariae in blood and urine in Egypt

A P da Silva Araujo 1876 found microfilariae in the blood in Brazil and used the name of Wucherer in connection with the terminology

J Bancroft 1876 found microfilariae in the blood in Brisbane, Queensland and sent specimens to Roberts urologist in Manchester In December 1876 he discovered adult filariae in ab cess of arm of a Chinaman in Brisbane and referred it to Spencer Cobbold in London who named it *Filaria bancrofti*

P Manson 1877 discovered microfilariae in blood of Chinese in Amoy China and described for the first time nocturnal periodicity and in 1878 the development of the parasite in *Culex fatigans* later found adult female *F bancrofti* in a lymph scrotum Repeated experiments on development of filaria in mosquito in 1883

A G Bourne 1888 described the male of *F bancrofti*

[Though most of the material in the following pages has been derived from Manson s Diaries and publications in *Customs Reports* the main conclusions were published and most of his figures and charts reproduced

in 1883 in a book The Filaria sanguinis hominis and certain new forms—A Parasitic Disease (H K Lewis London)]

Before recounting in detail the story of Manson s parasitological researches in Amoy China certain facts must be appreciated He was 33 years of age (Fig 2) recently married had two children and was living in complete isolation far from contact with any center of civilization in a



Fig 2 Patrick Manson Taken in Amoy South China October 23 1877 at the time of his discovery of the mosquito as tran m tte of *F lar a banc ofti*

small European community with a few kindred spirits (Fig 3) He had no one with whom he could discuss problems or work and none to whom he could turn for advice He had few works on parasitology and only a scanty medical library It should be remembered as Cobbold pointed out in his Parasitology in 1879 that the rather exaggerated claims made by da Silva Lima (1876) and da Silva Araujo (1878) in Brazil were due to the fact that neither had ever heard of Manson or his work in China *

When Manson wished to investigate some particular subject he was wont to consult Spencer Cobbold in London Timothy Lewis in Calcutta

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* Lima J F da Silva, *Ext at ida da Ca ta Med a da Bahia* (18 6) Araujo da Silva, A J P *Ibid* Ser 3 pp 106-109 (18 8)

or Leuckart in Leipzig—and it must be remembered that it took from four to six months to obtain a reply When engaged on work with mosquitoes he desperately needed some book on entomology He therefore approached the appropriate authority in the British Museum who belatedly replied that regretfully no work on mosquitoes existed but forwarded a treatise on the *cockroach* which he hoped might do instead! Indeed Manson wrote



FIG 3 Amoy South China 1873 showing location of Manson s hou e and laboratory on the island of Kulangsu (represented by arrow)

to Cobbold on November 27 1877 I live in an out-of the world place away from libraries out of the run of what is going on so I do not know very well the value of my work or if it has been done before or better

Manson s Original Work on Filariasis

Historical T R Lewis in Calcutta first discovered in March 1870 the parasite known as the *Filaria sanguinis hominis* in the chylous urine of a Hindoo Subsequently in many other cases of chyluria almost without exception he found this organism but toward the end of July 1872 he discovered similar filariae in the blood of a chyluria patient nine were present in a state of great activity in a single slide Later two other similar cases presented themselves with the same results Lewis suggested 400 000 as a reasonable estimate of the numbers of filariae in the patient s blood One patient two years after the appearance of chyluria developed ele

phantiasis of the scrotum while filariae could still be demonstrated in the blood Subsequently Lewis performed two autopsies on chyluria patients with parasitemia but after a prolonged search failed to find the parent worm It must be appreciated that helminthologists at that time knew that the filariae in the blood represented the young of some mature nematode So Lewis turned to the filarial disease of dogs which was so common in Calcutta and found it in ten out of the first twenty seven dogs examined It was associated with fibrous tumors along the walls of the aorta and of the esophagus and other minute nodules in the same situations When incised these were found to contain one to six mature nematodes of a pinkish sanguinolent tint ranging from 1 to 3½ inches in length and of both sexes

Applying these findings to the filariae of man Manson assumed that the mature nematode should be lodged in the walls or in the neighborhood of an artery vein or lymphatic and that through a rupture in the lining it poured its brood of ova or embryo filariae into the blood It was not possible to refuse to see in either of these two forms of filariasis the cause of the disease with which they are found associated? which parent or progeny could be the cause? He thought that the progeny were too small to cause any obstruction in either blood or lymph capillaries They had been found in the blood after all symptoms of chyluria had passed away and in cases in which there was no lymphatic disease As regards the parent nematode he thought otherwise because a great amount of local damage could be demonstrated He conjectured that in humans the parent parasite might be located in the thoracic duct or in the receptaculum chyle Moreover the geographic distribution accorded entirely with that of some allied diseases produced by parasites

The main parasite of the dog in China was *Filaria immitis* which infested about 50% In fifteen out of forty cases he found the embryos in blood taken from the ear The embryo measured 1/100 inch in length by 1/3000 inch in breadth On close examination something like a mouth could be distinguished at the blunt extremity In freshly drawn blood the animalcule was in constant motion wriggling about among the blood corpuscles like a snake Manson remarked that he had never observed any signs of growth or development The appearance and measurements remained constant Some idea could be formed of the vast numbers in the circulation and it was a matter of wonder how the host could continue to live and even maintain health and condition but he had watched dogs which had been thus infected for many years but yet appeared to be in no way inconvenienced The adult filariae were found coiled up in the right ventricle sometimes extending through the tricuspid valve into the auricle As a rule females were more numerous than males in the proportion of

2 1 Manson gave a careful description of the anatomy with special reference to the development of the embryo filariae from the ovarian cell and also made a similar study of the reproductive organs of the male

The story of *Filaria sanguinolenta* of the dog is treated in the same manner as to its anatomy and the disease phenomena to which it might give rise

III Discovery of *Filaria sanguinis hominis* in China

With the practice acquired in the dog Manson commenced systematic examination of human blood and found *Filaria sanguinis hominis* fifteen times To help him in this work which was excessively tedious and laborious he taught two Chinese assistants to recognize these hematozoa No selection of cases was made and soon he had records of 190 with the finding of filariae in 15 (about 8%) He found that *Filaria sanguinis hominis* closely resembled the canine hematozoon He also found that the short time during which the blood is clotting was the most favorable for minute examination

The organism was slightly less than 1/5000 inch in breadth and about 1/90 inch in length The human variety in contrast to the canine was provided with a very delicate noncontractile tegument within which the body of the animal is incessantly shortened and elongated This explains the lash of extreme tenuity being sometimes visible at others not The sheath followed like a limp string the movements of the body of the animal The last was the collapsed integument from which the head and tail had been withdrawn Manson noted in many specimens of the human filaria but not in all an elongated yellow patch in the center of the body He believed (correctly) that it appertained to an alimentary canal On close examination movements of the mouth which he compared to the breathing movements of a fish in the water could be discerned and he thought also that it was provided with lips In order to facilitate discovery instead of dividing the drop of blood onto six slides he had latterly been in the habit of placing the entire drop between two slides using one as a covering glass Thus equipped he began to speculate (1877) on the *development* of *Filaria sanguinis hominis*

Escape of the embryo from the original host The embryo in order to continue its development and keep its species from extermination must escape from its host in some way Manson hypothesized that after accomplishing this it either lived an independent existence during which it was provided with organs for growth or that it was *swallowed* by another animal which *treated it as a nurse* for such time as was necessary to furnish it with an alimentary system

IV The Mosquito Found To Be the Nurse

As the first step in the history of the *hematozoon* was in the blood the next might happen in an *animal which had fed* on that fluid To test this idea Manson procured mosquitoes which had fed on the blood of Hin Lo * (Hin Lo his gardener was infected with filariae he was very cooperative being always at hand and being in every way an excellent human guinea pig)

On examination of the expressed contents of the mosquito abdomen from day to day Manson found that his idea was actually correct and that the filaria which had entered the mosquito as a simple structureless animal left it after passing through a series of highly interesting metamorphoses during which it increased greatly in size possessed of an alimentary canal and otherwise equipped for an independent existence

History of the mosquito after feeding on human blood Manson s observations were made exclusively on the females of *one* species of mosquito He had never met with a male insect charged with blood

In Amoy he recognized two species during the summer one quite a large insect about half an inch long with black thorax and black and white banded abdomen (*Aedes aegypti*) the other about half that size of a dingy brown color (*Culex fatigans*) The former was comparatively rare the latter very common and the insect he had been studying

It took 2 minutes for the mosquito to fill itself with blood then it became so embarrassed by the weight of its distended abdomen that it could no longer wheel in the air Accordingly it attached itself to some simple surface if possible near stagnant water where it remained in a torpid condition digesting the blood excreting gamboge colored feces and maturing its ova This process was completed in the course of 3–5 days and the insect betook itself to the water to deposit its eggs On the surface of the water the eggs floated as a dark brown mass looking like a flake of soot The eggs did not take long to hatch (They are beautifully shaped objects like an Etruscan vase) and the embryos emerged by forcing open a sort of lid placed at the broad end of the shell The larvae then escaped into the water where they swam about and fed and soon became the jumpers we are all familiar with that are found in every stagnant pool

Manson s description of the anatomy of the mosquito When the mosquito had fed and after the food had been absorbed Manson distinguished the *following parts two ovisacs containing from sixty to a hundred ova* two large glandular masses intestine esophagus and a stomach—a very

* Correct name is Hin Lo in previous publications especially in the Life and Work of Sir Patrick Manson I have inadvertently referred to him as Hin To

delicate transparent bag If the blood in the dilated stomach was examined soon after ingestion the blood corpuscles could be distinctly recognized but changes occurred rapidly The corpuscles lost their outline and crystals of hematin appeared Soon corpuscles and crystals gave place to large oil globules and before the eggs were deposited all coloring matter had disappeared

Method of Procuring Mosquitoes Containing Larval Filariae Manson's most successful method of procuring filaria bearing mosquitoes and of preparing their bodies for microscopical observation were as follows *

He persuaded a Chinese (Hin Lo his gardener) who was heavily infected with filariae to sleep in what he termed a mosquito house (which he constructed out of chestnut wood as he described himself as a competent carpenter albeit an indifferent surgeon) in a room in which mosquitoes abounded When the victim had gone to bed a light was placed beside him and the door of the mosquito house was kept open for half an hour (The house was covered with fine gauze and the door was fitted with a spring hinge and opened inward) It was a large square wooden frame (10 × 10 × 6½ feet) In this manner many mosquitoes entered attracted by the light which was then put out and the door closed Next morning the gauze was covered with an abundant supply of engorged insects (Fig 4) These were then caught below a wine glass paralyzed by a whiff of tobacco smoke and transferred to small phials into some of which some water was poured A cover providing ventilation was then placed over the mouth of each phial The effect of the tobacco smoke was quite evanescent From the phials the insects could be removed from time to time as desired by again paralyzing them with tobacco They were seized by the thorax with fine forceps the abdomen was torn off and placed on a glass slide and a small cylinder such as a thin pen holder was rolled over it from the anus to the thoracic attachments to express the contents so that observation was not interfered with by the almost opaque integuments A thin covering glass (cover slip) was placed over the residue in which would be found the filariae either within the walls of the stomach or if these had been ruptured floating in the surrounding water

The first pregnant observation Manson made (which has since been confirmed) was that the blood in the stomach of a mosquito that had fed

* The results of this research in 1878 On the *Development of Filaria sanguinis hominis* and on the Mosquito considered as a *Nurse* were published August 31 1878 in *Journal Linnean Society* XIV Zoology No 75 pp 304 311 but were communicated by Dr Cobbold March 7 1878 The dates given in most textbooks and in the literature are wrong because the volume is labeled 1879 There is printed evidence that the journal was available in 1878

on a filaria infected man usually contained a much larger proportion of filariae than did an equal quantity of his blood From counts he made of blood from the finger and from the mosquito s stomach Manson concluded that the insect had the faculty of selecting the embryo filariae and he saw in this strange circumstance additional reason for concluding that this insect was the natural nurse of the parasite



Fig 4 Pictorial representation of Manson (August 1877) in his laboratory in Amoy South China performing his first experiments on transmission of *Filaria sanguinis hominis* He is transferring mosquitoes (*Culex fatigans*) which have fed during the night on the blood of Hin Lo his gardener who can be seen lying on a primitive couch in his mosquito house The infected mosquitoes are being put into bottles (see Fig 7) to be sent to Spencer Cobbold Note the spittoon The original of this painting is in the Wellcome Museum of Tropical Medicine in London

Not all embryos attained maturity and he found that the greater number disintegrated or were expelled in the feces At the end of the third fourth or fifth day when the stomach was quite empty and the embryos could not be easily overlooked very few (two to six) could be found in their original or slightly more advanced stages

Metamorphosis For a short period after entering the stomach of the mosquito the filaria embryo retained its original appearance and habits *It was a long snakelike animal with a transparent structureless body* enclosed in a delicate sheath but in a few hours changes commenced The tube or sheath separated from the body giving the appearance of a distinct

double outline and the body itself became covered with closely set transverse striations Oral movement could be distinguished and presently the sheath was either digested by the gastric secretion or cast off as a snake casts its skin Then the animal moved about unfettered and the striation became more distinct As the blood thickened so the movements of the embryo became less vigorous and the markings disappeared This the first stage of the metamorphosis took 36 hours to complete

Now the larva entered a sort of chrysalis condition during which nearly all movements were suspended and the outline and dimensions much altered The body became shorter and broader only the extremity of the tail did not participate in the change but continued to be flexed vigorously at long intervals meanwhile all oral movements ceased The body became sausage shaped Large cells occupied the previously homogeneous looking body and indications of a mouth appeared, if a little pressure was applied granular matter and cell like bodies escaped from an orifice placed a little in advance of the tail The animal now began to increase in length and sometimes also in breadth the growth seemingly taking place at the oral end of the body The mouth was four lipped the lips being open or pursed up From the mouth a delicate line could be distinctly traced passing through the whole length of the body to the opening (anus) already referred to near the caudal extremity At this stage some feeble movements might still be detected in the caudal appendix but when a certain length had been attained the tail gradually disappeared

Specimens of the filaria in the third and last stage were difficult to procure because most of the mosquitoes died on the fourth or fifth day after feeding and if their bodies fell into the water when examined they were found to be soft and sodden and without filariae which had either decomposed or escaped Sometimes however ovulation did not proceed rapidly and the mosquito survived to the fifth or sixth day or perhaps death did not occur as it usually does soon after the eggs had been laid and the insect then might survive for 2 or 3 days longer In such survivors the last stage of the metamorphosis could be studied Four to six days or longer were necessary for its completion So rare was this event that in hundreds of mosquitoes investigated Manson had been successful in finding filariae *in this last stage* in four instances only In one of these he had observed a number of embryos in regular gradation from the passive chrysalis to the mature and very active larva so there could not be any doubt about the relationship of these various stages * As far as he could

* Owing to his method of squashing the mosquito between two slides he imagined that all these stages were evolved in the abdomen and it was not till 1883 that he realized that metamorphosis took place in the thorax (see p 104)

make out the body gradually elongated from 1/100 to 1/30 inch but when mature the larva measured 1/15 inch in length by 1/500 inch in breadth At the last stage large cells occupied the interior and accumulated around the dark line (alimentary canal) running from the mouth to the caudal extremity In this manner the alimentary canal was fashioned and the peculiar and characteristic valvelike termination of the esophagus in the intestine as seen in filariae was developed Movements recommenced first a swaying to and fro becoming brisker The body gradually elongated and became slightly thinner and all cellular appearances vanished The extremity was slightly tapered and crowned with three perhaps four papillae used in *penetration* of the tissues of man on escaping from the mosquito

At this probably the final stage of the filaria s existence in the mosquito it became endowed with marvelous power and activity It rushed about the field forcing obstacles aside moving indifferently at either end and appeared quite at home in no way inconvenienced by the water in which it had just been immersed This formidable animal was undoubtedly the *Filaria sanguinis hominis* equipped for an independent existence and ready to quit its nurse the mosquito There could be little doubt thought Manson as to the subsequent history or that the filaria escaped into the water in which the mosquito had died and that it was through this medium brought into contact with the tissues of man and what was more probable that being *swallowed* it worked its way through the alimentary canal to its final resting place Arrived there the development was completed and fecundation effected Finally the embryo filariae that Manson had observed in the blood were discharged in successive swarms and in countless numbers In this way the genetic cycle was completed (See also Section XII)

V The Bearing of These Observations on the Explanation and Prevention of Elephantoid Disease

The statistics Manson had gathered amply sufficed to prove that the association of *Filaria sanguinis hominis* and elephantoid disease is one of cause and effect but now that he had shown that the mosquito was a necessary element for the perfection of the parasite the limitation and distribution of elephantoid diseases to certain districts and zones of the earth s surface received its explanation Such diseases were endemic only where the mosquito flourished as in tropical and subtropical climates Elephantiasis was more common in certain districts than in others and Manson ventured to predict that where the disease prevailed the appropriate mosquito would be found but that where the disease was absent or uncommon the mosquito would be found to be rare or to be represented by species incapable of nursing the infant filariae The habits of the people

especially with regard to the use of water and of mosquito nets would undoubtedly exert an influence on the amount and spread of filarial disease This was a point deserving investigation in view of the fact that this disease depends upon such a tangible link as the mosquito it should be quite possible to prevent its spread

VI Hypothesis on the Location of the Adult Human Filaria Parasite

The next consideration was the location of the then hypothetical adult parasite the parent filaria Manson *considered it necessary to determine* this before he could undertake any further far reaching research The integration of these various approaches revealed the cold logic of his mind

Subsequent to the finding of filarial ova in the aspirated lymph from a case of varicose groin glands he systematically set to work to ascertain whether in true elephantiasis any traces of embryos or ova could be found in these enlarged glands that accompanied this condition If this could be done it would supply irrefutable proof of the filarial origin of this phenomenon as incontrovertible as fossils in the rocks are of extinct races of plants and animals

In order to equip himself for this task Manson first examined the contents of the vagina and uterine tubes of *Filaria immitis* of the dog so as to acquaint himself with the appearance of the aborted eggs and their collapsed chorional envelopes subsequent to the escape of the embryo filariae When he had made himself familiar with these he concluded that he should be able to pronounce upon the nature of any debris which he extracted in gland lymph

Subsequently he found in almost all elephantiasis cases that a small quantity of lymph could be expressed into the hypodermic needle and that this contained collected into irregular masses bodies that might be connected with the parent parasite

In this conception he was sustained by T R Lewis who had decided that the filaria was viviparous

VII The Association of *Filaria sanguinis hominis* with Disease

For evidence of the pathogenicity or otherwise of his *Filaria sanguinis hominis* Manson *began to search around* for a series of cases of filarial disease especially chyluria lymph scrotum and elephantiasis He was evidently quite worried to get some explanation of the vagaries of this parasite He had noted as early as 1877 that its presence was not inconsistent with good health and he was puzzled as have been many since those days that in the majority of cases with the most marked expression of filarial disease

(i e disease actually caused by the parasite without any other concomitant infection) the filariae were absent from the blood stream This was indeed an anticlimax and he was at some pains to provide a reasonable explanation of the pathogenesis of what appeared to be an anomaly This is the underlying reason for the detailed histories of 53 Chinese patients with their blood findings which he recorded in the second notebook (1875–1877) The notebook consists of 182 pages devoted to a review of the literature of filariasis compiled in the British Museum Library in 1875 (inscribed B M March 30 1875) Many of the cases he referred to at this time were amplified in his paper entitled A further observation on *Filaria sanguinis hominis* in the *Imperial Maritime Customs Report II* (special series for the half year ending September 1877 pp 17 26) The observations spanned a period from May 18 to July 14 1877

By this time Manson had trained two Chinese assistants sufficiently not only to collect the blood specimens but also to carry out the microscopic examinations and he records carefully on each occasion the steps he took to check their results (He gives the names of his two Chinese assistants as Priah and Jak) The cases were all males with the exception of one female and from 21 to 61 years of age Special attention was paid to their history because he attached importance to previous attacks of fever of a lymphatic nature by this he meant lymphangitis and its differentiation from malaria which at that time was almost universal and amenable to quinine

The other dominant idea which runs through all these histories is the conviction that chyluria lymph scrotum and elephantiasis were all interconnected and might lead one to another The association of glandular enlargement in connection with elephantiasis he considered a prerequisite

These glands were commonly much enlarged and tender and in this he conceived the presence of the hypothetical adult worm in the lymphatics In this conjecture he proved eventually that he was not very far wrong It is almost impossible to give a statistical analysis of these numerous cases Suffice it to say that in three well marked cases of filarial disease with lymph scrotum enlarged varicose inguinal and femoral glands he found no filariae in the blood but in others they were present in association with ulceration of the legs twice with cataract of the eyes with ulceration of cornea with hematemesis with quartan fever with hydroceles recurrent inflammation of the scrotum with lymph scrotum enlarged glands elephantiasis of the leg with chyluria and in one case with no filarial disease but with extensive leprosy The exact pathological meaning of filariae in the blood puzzled him at the earliest stages of this research and the wonder is that he eventually was enabled to disentangle it

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[The complete series of cases of filarial disease from which this resume has been composed is to be found in the book he published in 1883 *The Filaria Sanguinis Hominis and certain new forms of Parasitic Disease* (H K Lewis London) as well as in the Imperial Maritime Customs Medical Reports (Series 2 for the half year ended September 30 1879 18th issue Shanghai 1880 pp 31 51)]

VIII Observations on the Filariae of Birds and Their Bearing on the Evolution of the Embryo in the Blood

In order to clarify his views on the production of embryos in the adult filaria and to explain the evolution of the embryo from the egg Manson turned to a study of the filariae of birds the results led him to believe that Lewis explanation of the process was probably correct Having so far failed to find the adult filaria in any of his human cases and being unaware of Bancroft s discovery of the adult female worm in Brisbane in 1876 he therefore had recourse to the filariae of some birds that abounded in the villages and gardens in the vicinity of Amoy The passages that follow are quoted or rephrased from the Diary it is necessary to explain that the term haematozoa which he commonly used refers to the filariae in the blood (now known as microfilariae) By diligently shooting many of the crows and magpies near his house he was able to find that a large proportion of the white necked crows (*Corvus torquatus*) acted as the hosts of two kinds of embryo filariae possibly belonging to different species (Fig 5)

The following is an extract from his Diary

28th October 1879

Yesterday afternoon at 4 30 p m I shot 3 crows and immediately examined the blood of one It was full of haematozoa This morning I examined the blood of the other two but found no haematozoa but this morning at 9 a m. I shot four crows and in the blood of one there were large numbers of haematozoa

He goes on to report that in both of these crows six to eight female parent worms were found in the first in the right ventricle and pulmonary artery in the second in the pulmonary artery alone The parent worms measured 1 inch to ¾ inch long by 1/90 inch broad They were alive and moving feebly Both ends were tapered down to about one third the diameter of the body The vagina was emitting live free embryos propelled by peristalsis of the uterine tubes and vagina about 1/100 inch from the mouth. The anus was situated near the extremity of the tail The mouth was simple and closed No male worm was found The embryos were shown in a sketch They were of two kinds—one larger and very active the other shorter and slow and languid The larger measured 1/100–1/120

inch by 1/5000 inch and were shaped like the hematozoon of the dog (*Filaria immitis*) with a pointed tail and very manifest movements When opened the mouth seemed to be surrounded by four papillae and a clear line could be seen running for a short distance down the body

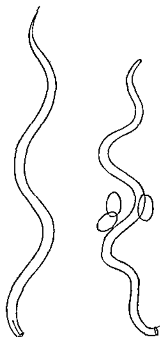


FIG 5 Original drawings of microfilariae of Chinese crow (*Corvus torquatus*) found by Patrick Manson Amoy China October 28 1879 and described in his Diary

The smaller embryos measured rather less (1/165 × 1/5000 inch) and were very languid The tail tapered slightly and then became abruptly truncated the head as in the larger embryo had a very distinct four papillated mouth extending from the head was a very delicate tube that looked as if it had been the skin of the worm in which the body had retracted Movement continued on oiled slides for many hours The sharp-

tailed embryo remained alive for 3 days but the truncated tailed one did not The smaller embryo was provided with a delicate enveloping sheath this extended anteriorly and posteriorly to form a lash at the head and tail With a *view of ascertaining the origin and nature of this sheath* Manson made many dissections of infected crows

In watching the birth of the embryo he found that as it approached the vaginal outlet by dint of energetic movements it gradually elongated the *delicate chorional envelope until the ovum from being almost globular* became oval Its poles were then pushed farther asunder until the contained embryo from being coiled up came to lie at full length with the walls of the chorionic sac so closely applied as to form a skin except at each *extremity Moreover during the process of elongation the membrane be*came so delicate that it could be discerned solely by careful observation. Manson was led to differ from Lewis in one respect that the process described above did not normally take place in the uterus or vagina of the parent but occurred *after the birth of the ovum during its passage* alon a lymphatic vessel He inferred that a similar process took place in *Filaria sanguinis hominis*

On November 1 1879 he records that he shot a magpie Hematozoa were found in the blood There were two species one measured 1/250 × 1/5000 inch the other about 1/100 × 1/5000 inch The former was languid the latter of active habit There were jerking pointing movement of the head that were quite distinct but the mouth was plain and no lash was visible at the head or tail Five parents were found in the magpie s right heart about 2½ inches long Two were males and were found in the pockets of the semilunar valves both aortic and pulmonary The female worms were 2½ inches in length the male measured ¾ inch with greate t breadth 1/115 inch and possessed double spicules with one or two minute papillae and a strongly incurvated tail The esophagus was straight 1/50 inch in length and terminated in the alimentary canal which ran parallel to the testis

Subsequently Manson came across one other instance of a sheathed embryo filaria in the blood of the handsome crowned pigeon (*Goura coronata*) he found this filaria to be almost indistinguishable from the filaria of man From observation of it he formed a speculation as to the manner in which the embryos maintain themselves in the blood current An excellent demonstration of the adhering power that the filarial embryos appeared to possess was seen in the blood of the Chinese grackle (*Gracupica nigricollis*) This bird was infected with two species of hematozoa The parent worms of one species lay coiled in the pockets of the semilunar valves (both aortic and pulmonary) Manson knew of the existence of the

other adult only through a study of its embryo which measured 1/90 inch by 1/4000 inch It had a truncated tail but no sheath and it displayed distinct oral movements Normally very active from time to time it fastened its oral extremity to the cover glass Its body could then be seen wriggling round and round this fixed point for minutes at a time While the filaria was fastened thus by the mouth the tail was applied and ap parently also fixed to the glass causing the body to struggle vigorously between its stationary extremities At this stage Manson related rather laconically it was the crows magpies and other birds which decided that this line of research must terminate They became so suspicious and wary that they persisted in remaining out of range

A list of species of birds and other animals that Manson examined (from October 1879 to January 1881) in the course of this enquiry follows

Chinese crow (*Corv s to quatus*)	2 species of filaria
Chinese magpie (*Pica edia*)	2 species of filaria
Ch nese minah (*Acridotl eres cristatel s*)	1 species of filaria
Chinese white grackle (*Grac pica nigr oll s*)	2 species of filaria
Tree sparrow (*Passer montanus*)	1 species of filaria
Kite (*Mil us melanoti s*)	1 species of filaria
Crowned pigeon (*Goura coronata*)	1 spec es of filaria

In addition he listed the paddy bird (*Herodias gar etta*) the yellow bittern woodpecker golden plover domestic fowl field snake mouse buffalo horse and cow—with a negative result

IX Nocturnal Periodicity

It had always appeared strange to Manson that *Filaria sanguinis hominis* had escaped observation in the blood for so many years till Lewis found it there in 1872 One would have supposed that with hundreds of workers in India and other parts the blood would have been searched thousands of times but notwithstanding this parasite which in some places is present in every tenth individual was overlooked for many years In 1877 Manson had remarked that in a filarious patient the embryos were frequently ab sent temporarily from the blood The explanation appeared to be that his examination was done in the *early* morning and of the two assistants he employed one worked during the day the other after 6 o clock in the evening

There is human drama here because one of these young men had a sick mother whom he attended with that filial concern so universal in Old China during the daylight hours so that he worked as hospital orderly during the night His companion came on duty during the day Manson re

marked that the day orderly made very few finds in comparison to the night orderly but was inclined at first to attribute it to accident However some months later he directed that the blood of a filarious patient be examined daily and that an accurate register be kept of the results On some days there appeared a great abundance of filariae on others none or very few he was not aware of any law governing this but recollecting the different results obtained by his assistants according as they worked during the day or after dark and now suspecting that it was not altogether accidental, he made a series of systematic examinations every few hours on this particular patient as well as on others with a view to ascertaining if this periodicity was maintained in every case In this manner he examined a number of patients and found that unless some disturbance such as fever interfered with the regular physiological rhythm the filarial embryos invariably began to appear in the circulation at sunset after this their numbers gradually increased till almost midnight but during the morning their numbers became fewer by degrees so that by 9 or 10 o clock in the forenoon it was a very rare thing to find any in the blood Then till sunset they appeared to have completely deserted the circulation but in the evening they came back once more to disappear in the morning and so on with the utmost regularity every day and also from day to day The cycle was completed every 24 hours and there were no longer spells of absence (as had been once supposed) than from morning till evening The figures he quoted abundantly sufficed to establish periodicity of the embryo s appearance in the blood The original chart of nocturnal periodicity (Fig 6) compiled by Manson is still preserved at the London School of Hygiene and Tropical Medicine It shows the readings made from Hin Lo s blood taken every

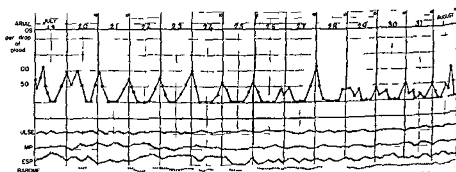


Fig 6 Patrick Manson s original chart of nocturnal periodicity made from 4 hour blood examinations of his gardener Hin Lo (1877) The curve of nocturnal periodic microfilariae as seen above and below are simultaneous records of pulse rate body temperature respiration and barometer readings (Original chart is in the London School of Hygiene and Tropical Medicine)

3 hours over a period of 14 days The ruling of the chart was made by Manson s own hand and shows great accuracy of execution

Ruminating on these points and on the readiness of the embryo filariae to discard their sheaths when removed from the blood he thought he should not have to look far for the explanation The nocturnal habits of *Filaria sanguinis hominis* were obviously regulated to the nocturnal habits of the *mosquito as its intermediary host* and presented another of the wonderful instances of adaptation so constantly met with in nature

[At this stage Manson thought of ascertaining whether the same sort of periodicity was observed by the flagellated organisms (*Trypanosoma lewisi*) discovered by Lewis in the blood of Calcutta rats He accordingly examined 30 rats in order to ascertain whether this organism occurred in China as well Six were found to be heavily infected There would therefore have been no difficulty in procuring subjects for observation but the trouble and cruelty of periodic examination of the rats blood were so great that he had to abandon the idea]

In reference to the facts quoted above several questions sprang to mind First was the disappearance of the embryos brought about by their death so that a fresh swarm had to be produced every 24 hours? Second if the embryos did not die where did they conceal themselves during the day? Third had this periodicity any pathological significance?

With regard to the third question he did not think it possible to provide an answer although it was possible to speculate vigorously His own conviction was that the true pathological significance in tropical disease was by no means fully understood nor was the importance of the parasite completely apprehended In order to obtain an answer to the other two questions as he was denied the privilege of postmortem examination of men he turned again to the dog and tried to get some light from the behavior of the embryos of *Filaria immitis* He therefore endeavored to ascertain whether there was a similar kind of periodicity in this parasite It became evident that there is a certain periodicity which is not quite so complete as in the case of the human filaria Embryos were never entirely absent from the blood though their numbers fluctuated and were always greater during the evening and night than during the day the period of greatest scarcity was from 9 00 A M to 1 00 P M This periodicity in the dog as in man had reference to the habits of the intermediary host This finding might be expressed thus As regards the embryo filariae in dogs there is in the daytime a *remission* in numbers in man an *intermission*

Hoping to get more information on the fate of the embryos during the period of remission Manson procured a large Chinese chow dog and for

a few days made a preliminary study of its blood parasites the creature however proved so unruly that the regular remission of the number of embryos became disturbed (as would happen from fever in humans) It appeared from this that embryos were at their lowest ebb in the early morning Accordingly he selected one morning when at 6 00 A M the embryos in a drop of blood numbered only 82 and administered a drop of prussic acid *The heart was found to contain four female and three male Filaria immitis*

Taking this in conjunction with the register of embryos free in the blood at different times of the day Manson concluded that the embryos of *Filaria immitis* do not die or disappear after such a brief existence as 24 hours but that they rest periodically in the most minute branches of the pulmonary artery and that when they disappear from the general circulation they are to be found in the lungs He was greatly puzzled to account for the method by which they managed to maintain their position in the blood current He conjectured that they attached themselves to the inner surface of the vessels using their oral extremities as a sucker, in a manner which he had observed when viewing under the microscope the human filaria a well as in those of birds There could then be little doubt that something similar happened in the case of *Filaria sanguinis hominis* and that during its period of temporary absence from the peripheral circulation the embryos rested in some of the thoracic or abdominal viscera awaiting sunset He concluded that a microscopic examination of the viscera of a filariated dog suddenly dying of a nonfebrile disease during the daytime would determine this point Manson gave statistics of the postmortem findings on two dogs *in both it was clearly shown that the greatest number of embryo filariae* were present in the lungs

Manson concluded that the parent filariae live in the lymphatics probably in the lymphatic trunks on the distal side of the glands They are oviparous and the eggs are carried by the lymph currents to the gland where they are arrested until hatched after hatching the embryo passes along the lymph vessels and enters the circulation Resting in some organ (probably the lungs) the embryos circulate with the blood during the night

X The Reversal of Nocturnal Periodicity

In December 1881 Manson investigated filarial periodicity still further in an attempt to disturb the rhythm in the manner of Dr Stephen Mackenzie s plan of turning day into night and vice versa (Mackenzie S *Lancet* ii pp 707 708 (1881)) The following information is taken from Manson s Diary The experiment is divided into three parts on three *subjects*

Subject 1 Jin male 25 years old a field worker The subject gave a history of an abscess of the lungs at the age of 12 to 13 and of a similar abscess when 17 years old in the right popliteal space Since that time he had had ague attacks in the autumn of an irregular character and often attended by inflammation of the inguinal glands Two men from his village had been operated for enlarged scrota but he had no knowledge of elephantiasis in his village of 200 souls He came to hospital on account of an enlarged spleen and dyspepsia A preliminary examination of his blood showed filarial normal nocturnal periodicity

Observations began in December 1881 Blood samples were taken from 6 00 A M at intervals of 3 hours day and night The maximum number of filariae per slide was 36 From December 9 to December 26 observations were made regularly For the first 5 days sleep was indulged in at the usual time During the remainder of the period the patient slept during the day but was kept awake during the night

Manson recorded data on the size of the blood drop on the slide the temperature in the mouth the number of filariae and the hours of sleep From the assembled data he constructed an accurate chart in which the night hours are blacked out by sepia paint from December 9 to December 23 1881

The total number of filariae during the night hours 9 00 P M -6 00 A M ranged between 100 and 120 It was clearly shown that the reversal of sleep began on December 15 and the periodicity became diurnal 4 days later on December 19

Subject 2 Teik Po male a farmer The subject was from a village 150 miles north of Amoy in which there were several cases of elephantiasis of leg and scrotum He had commenced to be ill four or five years previously with a tertian ague especially during the winter he had had an enlarged spleen for several years and for a year had suffered from an enlarged and painful testicle The spleen was enlarged to the umbilicus he was very anemic and there was swelling of the scrotum testicle and groin glands

Observations commenced on December 31 1881 and continued to January 24 1882 Teik Po slept 8 hours a day for 4 hours at a time i e 8 00–12 00 A M and 8 00–12 00 P M By this timing it was shown that the nocturnal periodicity had been disturbed and filariae began to appear in the blood during the daylight hours although the maximal numbers appeared from 9 00 P M to 6 00 A M This time no real reversal of periodicity took place and the correct term would be partially periodic Nevertheless the number of filariae was hardly comparable with that in the previous case The maximal number per slide was 28

Subject 3 In male 47 years old a farmer The patient came from a

village in which there were many cases of elephantiasis of the scrotum Since boyhood and nearly every year he had had swelling of the scrotum and testicles with fever lasting 3 or 4 days He had had tertian fever attacks with swelling of spleen and scrotum The groin glands were enlarged to the size of hen s eggs He also had right facial paralysis In came to hospital because of chronic ulcer of the right leg

Observations following the same lines as those on subjects 1 and 2 began on December 30 1881 and ended on January 26 1882 The reversal of sleep habits began on January 8 and filariae were found during the daytime 4 days later The results were similar to those in the foregoing cases The maximal number of filariae per slide in the blood preparations was 49

In a letter to the Editor of the *Lancet* in reply to one by Vandyke Carter of December 22 1882 Manson stated I have repeated Mackenzie s experiment of keeping a filarious subject awake all night and allowing him to sleep during the day and with the same result After two or three days, during which the ingress and egress of the embryos were very irregular periodicity became completely inverted

XI Manson s Search for the Adult *Filaria bancrofti*

No account of this pioneer work on filariasis could be complete without some reference to Manson s Herculean efforts to discover the adult filaria in human tissues From the start Manson was hampered in this quest by the bitter antipathy of the Chinese to examination of the body after death There are some intriguing stories of how he tried to evade this ban

Once early in August 1877 he recorded the following account although making no mention of the real circumstances in his Diary He knew of a Chinese who lived in a go down in Amoy native town and who was *sick unto death He also knew that for many years the man had been afflicted* with elephantoid swelling of the scrotum and legs and that he had filariae in his blood So Manson made a bond with the prospective widow for the sum of 200 silver dollars to be paid for permission to make a limited examination after death On a particularly hot day Manson accompanied by his favorite brother David (who practised in Takao Formosa) wended his way to the native town and found the body in a hot dark room Hardly had he finished his examination and dissection of the affected tissues than a loud hubbub was heard outside and through a chink they could see a milling mob chanting Death to the Foreign Devils The brothers just had time to gather up their *spoils and run for their lives* Eventually breathless they arrived at Manson s house disappointed and dejected without having achieved their aim

Manson s description of this case was discovered in his second note book It reads as follows Lien To male 34 a preacher unemployed At 30 he had general dropsy for one month a year later he had severe fever was delirious and very ill During the height of this fever the urinary flow diminished In appearance he was a large flabby man with coarse grained skin

On May 25 1877 numerous filariae were found in his blood The note on August 10 1877 says that latterly the patient had been bedridden and foolish in conversation and quite incapable of moving himself as he was so universally edematous It was noted that the transverse precordial dull ness extended from the nipple line to well over the right side of the sternum and that a soft systolic murmur became audible over the heart Throbbing of the vessels of the neck was noted but with a full regular pulse He died comatose on August 10 At autopsy filarial embryos were found in the blood from a small vein in the integuments of the thorax The spleen was enlarged and the liver cirrhosed a considerable amount of fluid was found in the peritoneal cavity The heart was dilated but no valvular lesion was seen There was a considerable amount of fluid in the left pleural cavity No *parent worm* was found in any vessel opened The thoracic duct was not found The examination was made under very dis advantageous circumstances —relatives crying wife in room and very obstructive atmospheric temperature 100 F no air very bad light much fatigue Not allowed to remove any part of the body

Nothing daunted Manson tried a second time In this case a Chinese patient died with extensive edema so Manson determined to search the body for parasites With the aid of a trusted old porter he smuggled the body to a piece of waste ground near the hospital where sheltered by a wall he excised a piece of muscle to look for trichinosis He found none but he had thought that on account of the native taste for pork this infection had to be excluded He found no evidence of filariasis either but then it dawned on him that a poor fellow he had attended ten years previously in Takao Formosa had really died from beriberi and not from real cardiac disease and this was just another similar case

Another attempt was made when he disinterred a corpse in the native cemetery to look once more for filariae but found an entirely new worm *Ligula mansoni* instead

Success came at last on October 11 1880 when he discovered the adult *Filaria bancrofti* in a lymph scrotum but Bancroft had beaten him by just four years

The patient Phie a male about 46 years old was a Chinese pedlar and



farmer with a lymphoid discharge from the scrotum The groin glands on each side were slightly enlarged and scanty microfilariae were present in the lymph In this instance Manson believed that the obstruction in the lymphatic circulation lay low down not higher than the femoral glands He judged it complete because of the scarcity of corpuscles in the lymph in addition to its pale clear character the absence of varicosity in the glands and the presence of embryo filariae in the lymph *but not in the blood* It was evident from the persistence of live microfilariae in this fluid that reproduction was continuous On October 13 1880 he removed part of the scrotum under chloroform mainly because of the danger to life of this continuous discharge of lymph He found the spleen much enlarged as well

The most affected part of the scrotum was dragged down until it was clear of the testicles and Manson removed a circular mass 2½ by 3 inches in diameter of soft spongy scrotum He then found that by pressing on the right femoral glands he could force a stream of lymph to flow with a projection of 3–4 inches On unfolding the cut surface he saw a long slender *worm wriggling vigorously in size and in color resembling* a piece of catgut but only about 2 inches of it were free He failed to extract the rest and the worm snapped off leaving its tail behind The head and mouth were simple and the vaginal opening 1/25 inch removed from the head It was packed with embryos in all stages of development and in those that escaped the sheath was quite distinctive Careful measurements were made of the worm itself and the organs it contained

On one more occasion he was near success In the latter days of Manson s active investigations in Amoy on the pathology of filarial diseases he had formed a theory on the blockage of lymphatic capillaries by the aborted ova of a dying or injured parent worm He had thought that this might be the underlying mechanism when he found filarial ova in aspirated lymph from enlarged groin gland when the embryos were absent from the bloodstream *The other condition that arose from the presence of* a defunct adult worm was abscess

On January 7 1881 Manson found the remains of a dead female filaria in an abscess of the upper part of the right thight in a Chinese who suffered from varicose groin glands In the dark yellow brown pus which exuded after incision portions of the filaria uterus *became entangled* in a needle he drew through the pus and in one fragment large numbers of fully formed outstretched embryos were demonstrated Embryo filariae were found in the night blood as well as the lymph aspirated from the groin glands

XII Confirmatory Data Regarding Filarial Metamorphosis

Those who have followed Manson's filaria story may be wearied by its length and the time it consumed (seven years) but this was not the end By 1883 the work appeared to be complete and was summarized in the book on *Filaria sanguinis hominis* that Manson published in that year In September 1883 we find him once more in a laborious undertaking to confirm his work of five years previously and to repeat it on a more extensive scale The records of this research which entailed dissection of seventy nine filariated mosquitoes with accurate measurements of the contained larvae occupy thirty six closely written pages of his Diary His main conclusions remained as before but a great many more details regarding the morphology and the development of the internal organs of the filariae are included and for the first time he realized the importance of the thorax

His systematic observations usually undertaken at 6 00 A M commenced on September 7 1883 and were continued till October 22 of that year

He sets his aims for reporting as follows (1) date of observation (2) species of mosquito (3) the number of hours after ingestion of blood (4) the quantity of blood in the abdomen (5) the state of the red blood corpuscles in the mosquito s stomach (6) the state of the ova in the mosquito (7) the number of the filariae in the abdomen (8) the condition of the filariae and state of their activity (9) size length and breadth of filariae in the thorax (10) markings of the filariae and observation on the sheath (11) special observations on the development of the filariae with reference to temperature

For instance after September 26 he noted that the nights became colder and the thermometer dropped to 75 F Subsequently his infected mosquitoes were kept in an incubator at a temperature ranging from 80 to 85 F and he noted that the rate of development of the larvae had become proportionately accelerated

In some the filariae were in greatest abundance in the abdomen where he counted hundreds and on one such instance only six could be found in the thorax In mosquito no 64 there were no fewer than 36 in the sausage stage in the thorax and in these he could recognize the formation of the alimentary canal and in the more advanced individuals aggregations of cells that might represent the vestiges of organs of generation

The most advanced and fully developed larvae (the infective stage) were discovered in mosquitoes nos 76 and 78 They were found in large brown individuals dissected 158½ hours after feeding on the gardener

Hin Lo One in particular, measuring 1/16 × 1/825 inch was in incessant motion in wriggling movements constantly coiling and uncoiling itself At the caudal extremity he could distinguish two if not three rounded papillae The alimentary canal could be seen moving about inside the *muscular body wall and accommodating itself to the movements of the body*

The final paper embodying this research was subsequently published in 1884 as *The Metamorphosis of Filaria sanguinis hominis in the Mosquito* (*Trans Linnean Soc Zool* 2 367 386 (1878 1886) with Plate XXXIX and numerous figures of the developmental stages of the larval filaria)

Remarking that this was the third occasion upon which he had repeated his mosquito experiments Manson undertook the task as he thought that he could do some service to my profession and to this branch of helminthology if I went over the ground I had trodden before He did so also to refute the criticisms of Lewis, Leuckart and especially Scheube (who saw no connection between the larval forms in the mosquito and the embryonic stages in human blood and even suggested that the filariae were digested in the intestine of the mosquito) It now became evident as Manson admits that he had been informed by R T Lewis of the penetration of the thorax by the larval filariae Lewis was the first to mention the migration Until I had read his description of his experiments on Filaria metamorphosis I entirely overlooked this significant point This word appears for the first time in the text as well as in the caption to the beautiful and accurate Plate XXXIX All the figures are accurate presentations judged by modern standards drawn by Manson himself and lithographed by C Bergeau It becomes apparent that he anticipated the fact that the larval filaria undergoes at least two ecdyses within the mosquito

In this study he dissected over a thousand mosquitoes and found in the filaria mosquito that 12–18 hours after feeding the thorax was full of parasites whereas those that remained in the stomach were digested He again distinguished between the different species and their capabilities of acting as a nurse The snuff brown one was undoubtedly *Culex fatigans* Of the tiger mosquitoes he recognized two the larger (*A aegypti*) was distinct from the smaller (*A scutellaris* now *A albopictus*) which had white bands on legs and body He concluded that neither was an efficient host In the mature larva he noted that the papillae spread out like the petals of a flower extending considerably beyond the margin of the circumference of the body

He also had evolved a technique for preserving the larval filariae on a slide by staining with gentian violet Finally he outlined a plan for concentrating fully grown larvae in bottles of water until a sufficient co-

centration had been obtained to infect man during the act of drinking and he entertained no doubt that the result would prove successful He apparently had stuck to this belief all through his research because a note has been found in the Diary which records an attempt to infect a Formosan monkey with *Filaria sanguinis hominis* on August 17 1878 On a Sunday morning a monkey was given 12 mosquitoes (full of filariae) on a plantain They had been seen feeding on Hin Lo s blood on the previous night The same number were given on the next four days The monkey s blood was examined on various occasions till November 3rd with a negative result

XIII Correspondence with Spencer Cobbold upon Subjects Already Discussed

Letter to Spencer Cobbold August 25 1880 on periodicity

Although in the paper I sent you some time ago I refrained from speculating on the cause of filarial periodicity yet I have thought a great deal about what might be the reason of this most remarkable phenomenon which savours of the marvellous As Dr Mortimer Granville remarks it will deserve the attention of physiologists For could we ascertain what the subtle influence is which sets these creatures circulating in the blood stream and arrests them with such military punctuality we probably would let much light in on many an obscure problem both in physiology and in pathology It was with the intention of providing myself with a standard with which to compare the results of observation and experiment that I prepared the chart I sent you

Dr Mortimer Glanville s ingenious speculations are based on the assumption that the phenomenon of periodicity depends in some way on sleep either on the mechanical changes in the circulation when the body is in the recumbent position or on the different proportions of oxygen in the blood or in the relative alterations of blood and tissue temperature during the waking and sleeping states Now as the embryos begin to appear hours before the usual time for repose and are in no way sensibly affected by changes in the hours of sleeping and waking it is evident that the power which fixes them and lets them loose operates independently of the sleeping state It is associated with the advent of night but not of sleep

According to Glanville The change of place may be fairly ascribed to change of state Looking to the habits of life in the lowest organisms it can scarcely be supposed that the periodicity can depend on the state or requirements of the Filaria It is not likely that the parasite needs repose or that it resorts to special localities to feed It seems more probable that the state of the circulating fluid determines the presence or absence of the Filariae in its main current by night and day respectively The first part of this I quite agree with but the last part I am not so sure about What is the difference between the state of the circulating fluid between 4 p m and 6 p m respectively? It is evident that something happens between these hours which liberates the embryos I do not know that physiologists have demonstrated or even supposed some sudden change beginning in the blood between these hours Again the conditions permitting the free circulation of the parasites continue with increasing effect up to midnight and the restraining influences which fix them are gradually supplied from that time till they effect almost complete

fixation by nine or ten o clock next forenoon What alteration in the physiological state of the blood or body generally corresponds to these hours? If you refer to my chart you will find no explanation in the rapidity of the circulation nor in the temperature of the body For sometimes the pulse is quick when the embryos are numerous and sometimes it is slow Sometimes the temperature fluctuates a degree without effect upon the numbers circulating

Whatever the cause may be certainly operates through the body the medium in which the parasites are but I very much incline to think that though operating through the body it is placed outside of it Of one thing we may be quite certain that from the fact of this periodicity being one of 24 hours its remote cause is the rising or setting of the sun or rather the altered relation of the earth s surface to the sun occurring every 24 hours Of one thing we may also be certain that the immediate cause is applied between the hours of five and seven p m What then i the phenomenon in nature which depending on the position of the earth s surface to the sun begins to operate on the human body with the utmost rigidity between the hours of 5 and 7 p m increases in power up to midnight wanes towards morning and finally ceases to act between 9 and 10 a m ? A correct answer to this would be a step towards the solution of this strange problem only a step however for the method of its operating would still remain to be explained We may dismiss at once the diurnal variation of atmospheric temperature and pressure for although especially in these latitudes these daily ranges are pretty constant yet when completely inverted as sometimes happens and as you may see from a comparison *of the chart and meteorological regi ter there is no corresponding disturbance* in filarial periodicity

In casting about for the answer two things occur to me 1st the rays emanating from the sun undergo about three hours marked alteration in their proportions and power 2nd they may make conditions of the earth s surface a change about the same time I incline to dismiss the former as the *direct* cau e for were the sun s rays the *direct regulati ig* influence we might expect to find the rhythm observed by the embryos affected by the presence or absence of clouds and so forth This is fa from being the case as you can see by comparing the chart with the meteorological register

The periodicity bears no relation whatever to the hours of sunshine cloud, or rain or other conditions influencing the quantity of kinds of rays impinging directly on the human body at least as far as I can see With terrestrial *magnetism the* case is quite otherwise Its variations are rhythmical If you consult authorities on the diurnal variations of the declination and inclination of the compass and intensity of terrestrial magnetism you will find a marvellous correspondence between the rhythm of these phenomena and that of filarial periodicity For example the needle of the compass crosses the magnetic meridian or mean daily position between the hours of 9 and 10 a m and of 6 and 7 p m during the night and early morning the north end of the needle i to the east of the meridian during the day to the west and hours when the meridian is crossed correspond pretty closely to the times of change from rest to activity and *ice ersa* of the filaria embryos

Again the *minimum* of daily change of terrestrial magnetic intensity is between the hours of 10 and 11 a m and the *maxi num* between 4 and 7 p m varying slightly with the season of the year These hours correspond very closely with hose of commencing rest and activity of the filaria in the normal state of the body There is no proof whatever that there is any cause and effect between the two phenomena

but the coincidence is most striking and suggests further investigation If experiment should how such relation it would be interesting to know if the cause operated directly or if the effect on the embryos depended on physiological changes in the body the result of terrestrial magnetism These may seem wild and unjustifiable speculations but I only offer them for what they are worth and desire to separate them by a clear and well defined line from my facts But the imagination has its place in science I believe as well as rigid inductive observation.

At any rate actuated by these speculations I have made one or two crude and unsuccessful experiments I wish very much some expert in physiology and electricity would take the matter up My knowledge is so limited and the apparatus I can command so crude that I despair of being able to give the answer myself I believe a systematic examination of the compound force called light, or of terrestrial magnetism on their influence on these worms would give most valuable result not only in solving this most interesting problem but in opening new and fertile field in physiology and pathology

I do not anticipate much from observations on the disturbing effect of drugs and the febrile state These undoubtedly in the future will be found to have an influence on filarial periodicity and it is possible this study may lead to just conclusions as to the cause of the phenomenon It is not likely however The conditions of experiment become in such cases almost too complicated to unravel We must be careful to bear in mind that substance in force which interfere with the periodicity may have nothing in common with its normal cause Assuming that quinine has this power it would be absurd to infer that the presence or absence in the blood of this drug has anything to do with normal periodicity It is only by the exclusion or inversion of the cause that we may hope to arrive at correct conclusion I have written more than I intended about my speculative subject The great interest you take in these matters is my excuse and I hope you admit it

I leave speculation alone now and pass to the facts in explanation of the chart I have sent you This chart records a series of observations on the blood temperature and on the pulse of two Chinese lads ascertained to be filarious and were in the main made by themselves After enlisting them in the cause and before commencing systematic observations I trained them to recognize and count the embryos with the microscope and to read accurately the clinical thermometer I took care from time to time to satisfy myself that observations were carefully made and recorded and I believe that if there are any errors in the chart, they are few and unimportant Observations were made every three hours day and night during one month At first the hours selected were 12 3 6 9 and 12 3 6 9 but after two days it was found convenient to change them to 1 4 7 10 1 4 7 10 The quantity of blood was as nearly as possible the same in each examination just sufficient to form a thin workable film fully occupying a covering glas 1/ × 1

The inevitable differences in the quantities examined probably account in part at least for discrepancies in the number of embryos found at corresponding hours on different days Notwithstanding this unavoidable imperfection the microscopical observations serve their purpose and in the main may be relied on The same clinical thermometer was used throughout and by both lad The instrument I find on comparison with two others is too high set by about a point of a degree This circumstance explains the range of normal temperature being in the 100th instead of the 99th degree as is usual

Food of the kind usually consumed by middle class Chinese viz rice a little pork

or beef salted and fresh fish and vegetables was taken at 7 a m 1 p m and 7 p.m. or thereabouts

Sleep during the night was constantly interrupted to take observations and consequently was frequently indulged in during the day The meteorological observations recorded in the chart were made with an ordinary large anaeroid barometer with thermometer attached The instruments though good enough perhaps for the purpose in hand are probably not perfectly reliable *I have accordingly procured* from the Customs a copy of their meteorological register corresponding to the period of these observations This is very accurately kept and may be trusted with the exception of the afternoon readings of the thermometer In consequence of the faulty *position of the instrument they stand 3 too high during the afternoon*

Both lads come from Hooie Oah a filariasis district some three days journey north of Amoy They have resided in Amoy a very few months Li Kha (I in the chart) is 27 years of age of average size and in good health He has no history of fever or any serious disease Tiong Seng (II in the chart) is 21 years of age, and is fairly well nourished When he was 14 had what he calls ague (what I call lymphatic fever) and from that time till now has on an average one attack about once a month These attacks begin with dizziness uneasiness of the body and limbs This gradually merges into a cold stage with moderate rigors of 2 to 3 hours duration then succeeds a hot stage of very high fever of 24 hours duration terminating in a moderate diaphoresis lasting for an hour or two The fever is accompanied by complete anorexia and during its continuation the inguinal and femoral glands invariably swell up and pain excessively Unless the attack of orchitis or inflammation of the tunica vaginalis to be hereafter alluded to he has never had any trouble about the genitals or limbs or any signs of elephantoid di ease The first three compartments of the chart refer to Li Kha I the second three to Tiong Seng II and the two lowest are accompanied by readings of their thermometer and barometer At the margin are numbers referring to numbers of filariae found temperature of the body beats of pulse per minute etc Along the top the figures refer to the date and the hours of the day

One or two things require a little explanation The effect of the *Febrile s ate* is well shown in the case of Tiong Seng (II) From the 12th of July when systematic observation commenced till the afternoon of the 16th he was in his usual hea h though his temperature ranged rather high and the filarial rhythm was perfect At 1 p m on the 16th after being out of sorts all morning he had a rigor followed by a rapid rise of temperature and smart fever At 4 p m he took 5 grains of quinine by 10 p m inflammation of the right tunica vaginalis with effusion and pe haps orchitis declared itself and the groin glands had become painful and swollen Next day he was confined to his bed inflammation continuing he took three doses of quinine of 5 grains each during the day On the 18th fever and inflammation had subsided and he took only two doses of quinine On the 19th the fever and inflammation relapsed and he had an attack of a sort of convulsive hysteria that day he had three doses of quinine On the 20th he was better and on the morning of the 21st was entirely free from pain and fever The swelling of the test cles and glands gradually subsided Contrast the behaviour of the thermometer and of the embryos *during and immediately after the attack* The disturbance in periodicity did not begin for some time after the thermometer had risen and it continued for days after the temperature had fallen to the normal standard Inference—the mere elevation of temperature has not *per se* any effect on the periodicity and it would show at once

were this the case Pathological changes consequent on the febrile state have and until these are eliminated or subside filarial rhythm is interfered with The effect of the fever seems to be to prolong the period of remission and to diminish the numbers circulating at the time of maximum and prevent complete fixation at any time

As the *qu nine* taken during the attack might have had some disturbing influence I tried the effect of a large dose in Li Kha (I) on July 26th Thirty grains were taken in three doses of 10 grains each at intervals of one hour beg nning at 10 a m On the following day you see the pulse rose the temperature fell and comparatively few embryos could be found circulating and their ingress that evening appeared to be delayed but by 1 a m on the 28th they were as numerous as ever and thence forward period c ty and numbers continued as before the experiment I cannot say however that th s slight perturbation was the result of the quinine for Tiong Seng (II) was treated in exactly the same way on the 29th but periodicity and numbers were in no way affected

Nitrite of amyl (15 drops) was inhaled by Li Kha (I) at 10 a m on the 25th There were no embryos in the blood when inhalation commenced shortly afterwards 2 were found on one slide 3 at 1 p m —? at 4 p m —0 at 7 p m and 18 at 10 p m

Santon n gr 4 was given to Li Kha (I) at 10 a m on the 29th and the same dose at 7 p m No effect apparent

Tu pentine spray inhaled by Li Kha (I) at 10 a m August 1st No result

Quassia tincture spray inhaled for 11 minutes at m dday August 1st by Tiong Seng (II) No result

Besides these I have tried one or two experiments with electricity but they proved barren and need not be deta led Referring again to your Quekett Club communica tion of 27th February I would a k you if Dr Bancroft has publ shed his observations on the dog louse as intermediary host of *Fila ia immitis* Unless he has observed metamorphosis of the embryo in the louse s stomach it is premature to conclude that this is the intermediary host Did the louse play the role he assigns to it then we might expect to find *F laria imm tis in the dog in all countr es* where this louse is found The intermediary host is I fancy the principal element in determining the geographical spread of such pa as tes A little reflection soon convinces one of this

Before concluding this letter I would suggest that Dr Somerville s statements about the habits of the Ch nese with rega d to the use of drinking water should not be received until he or someone else has given us the details of the investigations that have led h m to state that tl e Chinese do not drink uncooked water I have been many years in China and mix a good deal with the people and the outcome of my experience is that like most other people the Chinese drink water when they are thirsty and can get nothing better It i quite true that there is a certain class of Chinese which is p ejudiced against drinking cold water but it is only the rich classes who can afford to act on such prejudices I asked a Chi ese friend Do your countrymen often d ink cold water the farm servant and coolies? Certainly he said all drink water for if thirsty on the hill side or in the fields what else can they get to drink? Only a day or two after read ng Somerville s letter I asked con secutive patients as they passed through the Hospital consulting room about their drinking habits and thirst and the answers to my question Do you drink cold water?

1 Case of elephantoid leg a paper hanger Before my d sease began I drank cold water daily especially during the hot weather

2 A case of bruise in a boatman When thirsty I always drink cold water

3 Case of leprosy in a boatman When younger therefore before falling ill i.e. till I was 12 or 13 years old drank cold water in hot weather Since my leprosy commenced never drink cold water always tea

There happened to be nine lads assistants and dressers in the room when I interrogated these patients Turning to them I asked them individually if they drank water One and all confessed guilty of this habit and seemed very much astonished that any one should doubt it Foreigners are not so partial to water drinking as are the natives at least when water is drunk it is usually qualified with wine or spirits and aerated waters of different kinds are in general use

(This rather odd correspondence probably relates to Manson s belief that the larval filariae were conveyed to man in drinking water)

XIV Further Correspondence with Cobbold on the Fate of Experimentally Infected Mosquitoes

It is quite evident from the sense of Manson s writings in his Dairy and so well expressed in his correspondence that he harbored a great and enduring respect for the opinions of Spencer Cobbold the then President of the Linnean Society and generally acknowledged as the chief authority on helminthology of his age It is therefore with particular interest that we should pay special attention to the letter Manson wrote to him on June 20 1879 in which he informed him of the dispatch of a box from Amoy to London containing filaria impregnated mosquitoes preserved in glycerin so that Cobbold should be able to verify the truth of Manson s great discovery by dissecting them himself (Fig 7) It must be remembered that at this time the technique of sectioning insects in celloidin had not been discovered and that the paraffin method had proved unsuccessful In his correspondence with Manson Cobbold had suggested that he should communicate this confirmation to the Linnean Society in 1884 but he apparently never did so

The following story sheds a different light on this episode In 1935 Professor R T Leiper received a message from the curator of the Museum of the Royal College of Surgeons in London informing him that a quantity of helminthic material had been discovered there and requesting his assistance in identification Among other specimens was a cedarwood box addressed in Mrs Manson s hand Inside were found a number of phials each labeled in Manson s familiar handwriting containing filaria infected mosquitoes preserved in glycerin Each phial had been corked and sealed with paraffin wax and bound securely with a leather cap and cord The whole series was neatly fitted into pockets in a leather case of Chinese workmanship Obviously Cobbold had received it and had put it to one side unopened Leiper opened several bottles and the contained mosquitoes were found intact and on dissection were proved still to contain recog

nizable larval filariae (after an interval of 56 years) and furthermore they were identified by Edwards of the British Museum as type *Culex fatigans* At the same time Manson appears to have despatched some similarly pre served insects to Stephen Mackenzie in London as he had recently evinced some interest in filarial disease and had in 1881 studied filarial periodicity at the London Hospital and had succeeded in reversing it We do know that Mackenzie had preserved them because in 1896 Manson found one of



FIG / Mosqu toes experimentally infected with filaria by Manson and sent by him to Cobbold n 1879 Extract f om Manson s letter (June 20 1879) I will forward you by this mail filaria impregnated mosquitoes They are pre er ed in glyce ine and were fed on the blood of the man whose case I append I hope you ll pardon this delay in send ng the mosquitoes being n ge eral practice here the many interruptions th s entail make wo k of th s sort exceed gly diff cult to carry out quickly For full story of this ncident see text

his bottles in Mackenzie s house in Cavendish Square while preparing the Goulstonian Lectures on Malaria for the Royal College of Physicians Moreover Manson mentioned this occasion in a letter to Ronald Ross dated December 23 1895 as follows

Many years ago I sent Cobbold a lot of mosquitoes I knew that Cobbold s collection had gone to the Royal College of Surgeons so got perm ssion to look over them for my mosquitoes but failing * I we t to Stephen Macken e s house and there

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A derangement in the health of the human host affects the regularity of the periodicity in the appearance of the embryos in the blood Fever especially has this effect and men with or without, elephantoid disease are very liable to attacks of fever The question suggests itself what becomes of the embryo filariae during the time they are absent from the blood? Are they voided after finding their way into the excretions? No! I have failed to find them there Are they disintegrated in the blood or do they pass like leucocytes out of the capillaries into the tissues the parent emitting a fresh swarm every 24 hours? Their enormous numbers and the small size of the parent render this improbable To get an answer to this I turned to the analogous parasite the *Filaria immitis* of the dog By frequent observation of the blood of filarious dogs I found that the embryos of *F immitis* observed a certain though less marked periodicity and that from the hours of 9 a m to 2 p m they were much less abundant than at other times—that a drop of blood from a dog about 11 a.m might contain upwards of 40 embryos whereas at 9 p m a drop from the same might contain upwards of a thousand. I killed a dog whose blood contained 526 embryos in every drop At one time when the number of embryos had fallen to 8? per drop and examined the viscera with this result

One drop of blood exp essed	from spleen	32 embryos
	from kidney	0
	from liver	3 4
	from lungs	4582

From this I infer that at certain times the embryos of *F la ia immitis* of the dog rest in the lungs and perhaps the liver (probably this period of rest has reference to the habits of the intermediary host)

An analogous thing undoubtedly takes place in the case of the *Filaria sanguinis* of man but being deprived of the privilege of a *Sectio cadave s* in a suitable case I cannot say in what organ or t ssues their rest is made It is ju t possible that the fact of some particular part of the body be ng selected by the embryos as a resting place may give the key to diseases the parasite produces Where does the parent parasite live? Bancroft n Austral a and Lewis in India have found it in abscess of the limbs in hydroceles and in Lymph Scrotum but they have not been able to state positively in vhat particular structu e or tissue it resides From some recent observations I am able to state that the parent paras te has its habitat in the lymphatics that the embryos pass along the lymphatic vessels and thus through the thoracic duct, they reach the circulation If a ubcutaneous syringe is introduced into the varicose lymphatic gland so common in filarious pat ents and by its means lymph is drawn from these glands the lymph is found to contain embryos in much larger proj ortions than in the blood of the same subjects The inference from this is that the embryos pass along the lymphatics before reaching the general c rculation It may be objected to these conclus ons that these embryos find their way from the blood into the lymphatics This seem very improbable and I have exam ned a case lately which ent rely does away with this object on and proves conclusively that the parent lives in the lymphatics In the case of filarious blood in wh ch the groin glands were much enlarged and almost solidified I drew from one of the softest of the glands a few drops of clear watery lymph In th s lymph I found eleven o a containing live embryo and one embryo free These ova could

I found a small bottle with a solitary mosquito floating in glycerine On section of *the blood in the abdomen there were my Amoy filariae and in its thoracic muscles* they were too most beautiful to behold.

XV Correspondence with Dr Leisrink of Hamburg

The correspondence is cited from Manson s Diary

Hamburg 29th June 1879

Dr Med Manson Esq Amoy (China)

Dear Sir

I beg to take the liberty of asking you a favor by the accomplishment of which you will much oblige me In connection with Mr Professor Esmarck of Kiel I am about to write a work of inborn Elephantiasis (*Elephantiasis Arabum*) It is with the highest interest that we learn of the recent scrutinies partly originating from you concerning the filaria and its relation to Lymph Scrotum You would render us a great service by communicating *your present opinion of the situation* of elephantiasis scroti to filaria

Further whether there is also found filaria in the elephantiasis of arms and legs? What part the mosquito has in transplanting the sickness and are the *embryos* of filaria found in water (to drink)?

Further we should be very glad to be made acquainted with the statistical proportions of elephantiasis and if you would give us a description and perhaps if it would not cause you too much trouble an *image* of elephantiasis mammae

Of course we should in publishing our work thankfully indicate the sources from which our information in regard to the above questions are derived

By informing me about this matter you will indeed much oblige

Dear Sir
Yours faithfully
Dr Med H Leisrink

Amoy 12th August, 1879

Dr Med H Leisrink

I have obtained some additional evidence for the parasitic origin of elephantiasis and made out one or two points of great significance and importance in the life history of the *Filaria sanguinis hominis*

You will observe that in my papers on the development of the *Filaria sanguinis hominis I state that the embryos are sometimes present in* the blood of the human host sometimes absent I had made out that no law regulated their appearance and disappearance but now I know that their presence is regulated in accordance with the habits of the mosquito—their intermediary host Unless in *exceptional* circumstances the embryos are entirely absent from the blood from early in *the morning* till unset. When the sun sets the embryos suddenly appear in the blood and the mosquito in the air When the sun rises the embryos gradually leave *the blood*

promise to Manson that he would confirm his great discovery Indeed we know that Manson instructed him most minutely how by dissolving out the glycerin *from* the bodies of the preserved mosquitoes in warm saline it was possible to dissect them and *so to demonstrate the contained filarial* larvae

About 9 or 10 a m it is impossible or nearly so to find one I have most carefully worked it out and now accept it as fully proved. The observations will be published in detail this autumn.

A derangement in the health of the human host affects the regularity of the periodicity in the appearance of the embryos in the blood Fever especially has this effect and men with or without, elephantoid disease are very liable to attacks of fever The question suggests itself what becomes of the embryo filariae during the time they are absent from the blood? Are they voided after finding their way into the excretions? No! I have failed to find them there Are they disintegrated in the blood or do they pass like leucocytes out of the capillaries into the tissues the parent emitting a fresh swarm every 24 hours? Their enormous numbers and the small size of the parent render this improbable To get an answer to this I turned to the analogous parasite the *Fila ia immitis* of the dog By frequent observation of the blood of filarious dogs I found that the embryos of *F immitis* observed a certain though less marked periodicity and that from the hours of 9 a m to 2 p m they were much less abundant than at other times—that a drop of blood from a dog about 11 a.m might contain upwards of 40 embryos whereas at 9 p m a drop from the same might contain upwards of a thousand I killed a dog whose blood contained 526 embryos in every drop At one time when the number of embryos had fallen to 8? per drop and examined the viscera with this result

One drop of blood expressed from spleen	3	embryos
from kidney	0	
from liver	324	
from lungs	458?	

From this I infer that at certain times the embryos of *Fila ia immitis* of the dog rest in the lungs and perhaps the liver (probably this period of rest has reference to the habits of the intermediary host)

An analogous thing undoubtedly takes place in the case of the *Filaria sanguinis* of man but being deprived of the privilege of a *Sectio cadaveris* in a suitable case I cannot say in what organ or tissues their rest is made It s just possible that the fact of some particular part of the body being selected by the embryos as a resting place may give the key to diseases the parasite produces Where does the parent parasite live? Bancroft in Australia and Lewis in India have found it in abscess of the limbs in hydroceles and in Lymph Scrotum but they have not been able to state positively in what particular structure or tissue it resides From some recent observations I am able to state that the parent parasite has its habitat in the lymphatics that the embryos pass along the lymphatic vessels and thus through the thoracic duct they reach the circulation If a subcutaneous syringe is introduced into the varicose lymphatic gland so common in filarious patients and by its means lymph is drawn from these glands the lymph is found to contain embryos in much larger proportions than in the blood of the same subjects The inference from this is that the embryos pass along the lymphatics before reaching the general circulation It may be objected to these conclusions that these embryos find their way from the blood into the lymphatics This seems very improbable and I have examined a case lately which entirely does away with this objection and proves conclusively that the parent lives in the lymphatics In the case of filarious blood in which the groin glands were much enlarged and almost solidified I drew from one of the softest of the glands a few drops of clear watery lymph In this lymph I found eleven ova containing live embryo and one embryo free These ova could

not have come from the blood but must have been emitted by the parent directly into the lymphatics They measured 1/500 *inch* × 1/750 *inch* *This proves also that* this *Filaria sanguinis hominis* is oviparous

I trust you will excuse me writing thus at length on the subject of filaria without referring to the object of your letter—the relation of elephantiasis to the parasite. But as I consider the two related as cause and effect and that I can prove it these facts I have alluded to become of importance to you

Filaria embryos in the blood are not more frequently found in fully developed elephantiasis than in other conditions of health or disease At first sight this would seem to disprove the connection of filariae with elephantiasis completely But *although the embryos are not found in completely developed elephantiasis I find* them in many cases of *d veloping* elephantiasis and I find elephantiasi proper associated with diseased conditions in which the embryos are constantly or generally present.

Thus lymph scrotum is invariably or nearly so associated with the parasite and the embryos are frequ ntly and easily found in the lymph from the scrotum a well as in the glands and usually in the blood

Many cases of elephantia is scroti give a previous history of lymph scrotum Lymph scrotum is sometimes found in connection with elephantiasis of leg and scrotum Again I have a series of cases of partially developed elephantiasis of the leg with varicose groin glands exactly like those of lymph scrotum in which embryos were found either in the lymph from the gland or in the blood or in both The condition of lymphatics nece sary for the de elopment of elephantiasis appears to be complete obstruction of the lymph vessels of the parts—and the condition of course renders it impossible for the filaria embryos to pass along them rd to nter the blood Thi state of affairs is inimical to the parent worm and cau es its death In lymph scrotum and elephantia is with partially varicose glands there must be some cir ulation of lymph otherwise the gland would solidify and contract as we find them in pure elephantiasis Consequently the embryos gain ac ess to the circulation in these diseases

These remarks apply to elephantiasi of arms mammae and other regions as well as that of the scrotum and leg At present my conviction i that occasionally the *Filaria anguinis hom nis produces no di ease* whatever that usually either from its young a ting as emboli or as irritants it causes obstruction or inflammation of lymphatics connected with the vessel or gland the parent is residing in that th s obstruction once established is permanent and in its effects progressive independently of the life or death of the original cause ju t as a mechanical lesion of the valves of the heart the valves of a vein the contraction of a cirrhosed liver and so on abide and increase long after the disappearance of the cause that if the obstruction is complete and there is no circulation of lymph the lymph solidifies (ju t as blood in a ligatured aneurism) and *elephantiasis arab um* is the result that, if the *obstruction is only partial* there i vari osity of the lymph ves el and lymph scrotum chyluria or a lymphorrhagia of the leg may be the re ult in *this last* case filarial embryos are generally to be found in blood or lymph in the case of complete obstruction they are absent and probably the parent worms have long been dead killed by the solidifying lymph

Additional arguments for the parasitic origin of elephantiasis are to be *found* n the geographical distribution of the disease its limitation to particular districts These di tricts being always filarious the frequency with which a filar ou blood

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Additional arguments for the parasitic origin of elephantiasis are to be found in the geographical distribution of the disease its limitation to particular districts These district being always filarious the frequency with which a filariou blood

patient or a *lymph* scrotum or chylur a case comes from the same village or even house with an elephantiasis case

The attacks of erysipelas to which *elephantiasis cases are subject,* and which are often set down as the cause of the disease are only what one expects *to* find in cases of lymphatic obstruction or slight injury to the limbs or on the occurrence of fever That they are *not* the cause of elephantiasis is easily proved by the fact that there are many cases *of* elephantiasis in which fever or inflammation of the part have never occurred With regard to the part taken by the mosquito in transplanting the sickness I can only say that I have gone over the ground described in my paper on the subject and have found nothing to add or alter and that recently most of my observations on the mosquito as intermediary host have been confirmed by Lewis of Calcutta His observations appeared as an appendix to the Fourth Annual Report of the Sanitary Commissioners with the Government of India 1877

The mosquito is as necessary for the spread of Filarial Elephantoid disease as is the cow for the spread of tapeworm in man The embryo of the filaria after leaving the mosquito is so minute only 1/30th of an inch in length that to search for it in the water would be a *hopeless task* But the history of the mosquito makes it certain or nearly so that the embryo after going through developmental changes such as I have described quits the mosquito for the water I have seen it swimming powerfully in a drop of water into which it had been expressed from the abdomen of a five days old mosquito and no doubt could be entertained but that it was in its proper element

I do not know if I could furnish you with vital statistics of the various forms of elephantiasis of any value

There is no registration of death or disease in China and what I could give you would only be the cases I *have* seen in hospital but excellent statistics of the disease as found in Travancore and Cochin *are given by* Indian *writers to* whom I would refer you

I have only once seen Elephantiasis mammae and this *not* a well marked case Elephantiasis of the clitoris I operated on once removing a tumour weighing over nine pounds I think Elephantiasis of the arm is very rare I have seen it twice only Elephantiasis of the cheek *or* a disease like elephantiasis I have seen and operated on once

The operation I have described for elephantiasis I have *no*[w] practised many times with only three deaths and I can with some reason recommend it Esmarck's bandage I have tried but it is no use in constricting the pedicle at the final and most dangerous steps of the operation *But* I use it in elephantiasis of the legs which when of great size I frequently reduce with excellent results by dissecting off superabundant folds of hypertrophied tissue in such a case without a bandage—*an operation which would otherwise be difficult bloody and dangerous*

Apologising for this long letter and wishing you every success in your work

Yours etc etc

Patrick Manson

XVI Extract from Manson s Diary Recording the First Experiment on Filarial Metamorphosis in 1877

These data are historic as recording the birth of modern Tropical Med icine and are inserted here with this object in view

Citation from page 501 of Diary

Hin Lo brought me four mosquitoes which he had caught this morning in his mosquito net and which were distended with his blood I examined them this morning

No 1 Blood corpuscles not distinct Plenty of oil globules in the old digested blood and several cylindrical bodies of a pale grey colour and distinct outline These were about the size and might have been embryo filariae dead no movement.

No 2 Blood corpuscles also digested and two bodies one of which had a distinct to and fro movement of the head half of body and the appearance of ciliary current at mouth

No 3 and 4 Blood corpuscles distinct—in both live active filariae and in one of them 10 specimens—5 in a single field Differed from those in the man s blood in being perhaps more active Tail not well seen anterior head loop often very distinct, oral movements very apparent. Perhaps an oesophagus developing Double outline and transverse striation on the integument most distinct

Citation from page 503 of Diary

Observations on mosquitoes which had fed on Hin Lo s blood Blood expressed from insects abdomen

1st day Morning after feeding Blood corpuscles having no outline but distinct from one another Some filariae have a very distinct double outline with head and tail loop distinct and marked transverse striation

2 p m Mouth movements very distinct Striation most marked Movements active in most languid in some—none in a very few

10 p m Movements very languid Distinction of outline lost Mouth very indistinct Body speckled with granules of different sizes

2nd day 7 a m Many specimens dead most languid One more active showed oral movements with a distinct transverse striation and inside the body of the animal a distinct to and fro movement of the dark and shining granules as if in a fluid contained in a tube

XVII Manson s Correspondents on Filariasis

A Timothy Richards Lewis (1841–1886)

Timothy Lewis was a Welshman born in Carmarthenshire He was educated at a private school in Narbeth a *small town in Pembrokeshire* At first apprenticed to a local apothecary he came at the age of 19 to London to the German Hospital at Dalston He finally qualified at Aber deen in 1867

Already he achieved distinction as a student In 1868 he entered the Army Medical Service After a brief period at the Army Medical School

at Netley where he ranked first he was sent to India with his great friend D D Cunningham to investigate cholera having previously been trained in bacteriology on the Continent by Pettenkofer and others Reaching Calcutta in January 1869 Lewis and Cunningham became attached to the Sanitary Commissioner with the Government of India This team of two studied cholera and other Indian diseases for some twelve years and during this time published a series of important papers

Lewis was married in 1879 and returned to England in 1883 Subsequently until his untimely death in 1886 he held the appointment of Assistant Professor of Pathology at the Army Medical School Netley

Most of Lewis s original work was published in Appendices to the *Annual Reports of the Sanitary Commissioner with the Government of India* Some also appeared in contemporary issues of the *Quarterly Journal of Microscopical Science* His first paper concerns objects found in cholera stools and contains the first authentic account of amebas from the human intestine (1870) In 1872 appeared his famous paper on a haematozoon inhabiting the human blood and its relation to chyluria which contains the first account of *Filaria sanguinis hominis* The next contribution in 1874 was a description of filariasis in dogs it discussed the relation of filarial parasites to chyluria and elephantiasis Next with Cunningham he wrote on Madura foot and other fungus diseases and then they described oriental sore as observed in India In 1878 he published a memoir on microscopic organisms found in the blood of man and animals and their relation to disease this memoir contained an account of the spirochetes of relapsing fever and the first description of the trypanosome of the rat now known as *Trypanosoma lewisi* Lewis place in tropical medicine is secure He was indeed a pioneer and it is only just and fair that as Dobell suggested he should bear the title of the godfather of tropical medicine Manson was first in the field but it was Lewis who first made the fundamental discovery in filariasis Lewis was an indefatigable worker conscientious and careful and always cautious and clear in interpretation

B Thomas Spencer Cobbold (1828–1886)

Cobbold was born at Ipswich on May 28 1828 and died on March 20 1886 at Maida Hill London

He was first apprenticed to Cross the Surgeon of Norwich then he went to Edinburgh where he worked under Goodsir and Edward Forbes taking his degree in 1851 He was then appointed Curator of the Anatomical Museum In 1858 he removed to London and lectured on botany zoology and comparative anatomy at the Middlesex Hospital Medical School where he devoted himself mainly to the study of helminths From 1865 on he

engaged in the practice of medicine in which he persisted for ten years but keeping in touch with scientific progress He lectured also on veterinary helminthology until a few months before his death

He was elected a fellow of the Royal Society in 1864 in recognition of his excellent original work To workers in the tropics Cobbold became the high priest of helminthology so that men like Bancroft Myers Baelz and Manson referred their discoveries to him for description and identification and it was Cobbold who conferred the *imprimatur* by naming them after their discoverers

Cobbold is credited with the publication of some five hundred memoirs and papers on a great variety of subjects of this immense material some 108 papers deal with helminthology and they survive as a monument to his originality and industry His well known book Entozoa an Introduction to the Study of Helminthology with reference more particularly to the Internal Parasites of Man appeared in 1864

The best appreciation of Cobbold s scientific work is that by Brumpt in 1900 (*Arch parasitol* **3** 163 176)

C Rudolph Leuckart (1822–1898)

Leuckart was born on October 7 1822 at Helmstedt Brunswick and died on February 6 1898 at Leipzig In 1847 he became Privat Docent at Gottingen from 1850 to 1869 he was Professor at Giessen and from 1870 to 1898 he held the chair at Leipzig He became especially distinguished for his work on helminthology following in the wake of his uncle Professor F S Leuckart (1794–1843)

His first work on cestodes appeared in 1848 He then wrote on the Linguatulidae and subsequently on *Trichina spiralis* which had been discovered by Sir James Paget when a student One of his last and most important discoveries was the part played by *Limnaea pereger* as the intermediary host of *Fasciola hepatica*

Most of Leuckart s original work is to be found in his Menschliche Parasiten which appeared in 1863 and was translated into English

His Bibliography is given by Ray Lankester (1901) in Obituary Notices of Fellows of the Royal Society

XVIII References on Filariasis

References to Manson s early papers on filariasis cited in this paper

Patrick Manson M D

(1872) Customs Gazette No xiii January March 1872 Pt VI Medical Report for the Half year ended 31st March 1872 No 3 of the series Drs Muller and Manson s Report on the Health of Amoy pp 22 33 On the Operative Treatment of Elephantiasis scroti

(1874) *Ib d* Medical Report for the Half year ended 30th September 1874 No 8 of the series No XXII Pt VI July September 1874 Drs Jones and Manson s Report on the Health of Amoy for the half year ended 30th September 1874 pp 67 70 On Lymph Scrotum

(1875) China Imperial Maritime Custom Medical Peport for the Half year ended September 1875 No 10 of the serie Remarks on Lymph Scrot m Elephantiasis and Chyluria pp 1 14

(1877) *Il d* No 13 of the series Pt VI Custo ns Gazette XXXIII January March 1877 Report on Haematozoa pp 13 38

(1878) *Il d* II Special Series No 2 14th Issue Further Observations on *F lar a sanguinis l ominis* pp 1 26 with Plate Figs 1 16 showing development of *Filar a sang in s l o inis* in the mosquito

(1879) *Ibid* II Special Series No 2 Medical Peports fo the Half year ended 30 September 1879 Additional Notes o *F laria sang i n s hominis* and F laria Di ease at Amoy pp 31 51

(1880) *Ibid* II Special Ser es No 2 Med cal Reports for the Half year ended 30th September 1880 Is ue 20 Additional Notes on *Fila i sanguinis l o inis* and Fila ia D sease Discovery of Adult *F la c ofti* pp 13 15

(1881) *Ibid* II Spec al Series No 2 Med cal Peport for the Half year ended 30th Septemb r 1881 On the Periodic ty of Filarial Migrations to and from the Circulation (A letter to Dr Cobbold) With chart pp 63 68

(1882) *Ibid* II Special S ries No 2 Medical Reports fo the Half year nded 31st March 1882 Issue 23 Notes on Filaria Disease With 3 charts pp 1 16 Reversal of Nocturnal Periodicity

XIX On *Distoma ringeri (Paragonimus westermani)*

Paragonimus westermani (Kerbert 1878 Braun 1899) syn *Distoma ringeri* (Cobbold 1880) This fluke was first discovered by Kerbert in 1878 in the lungs of two Bengal tigers which had died in the Hamburg and the Amsterdam Zoological gardens

On April 24 1880 Manson found large numbers of operculated eggs in the rusty blood flecked sputum of a Chinese patient (Tso Tong) who had lived in Formosa (see below for details) In the previous year he had in hospital in Amoy a Portuguese suffering from aneurysm This man re turned to Tamsui Formosa and died suddenly in June 1879 Dr B S Ringer who made a postmortem wrote to Manson that he had found a parasite of some sort in making a section of the lung He thought that it had escaped from a bronchus

When Manson discovered the ova in the sputum he recollected Ringer s parasite and that the Portuguese patient had lived in Formosa so he con cluded that Tso Tong s lungs probably contained a similar parasite Ringer then sent him the solitary specimen preserved in spirits of wine Manson placed some of the sediment from the bottle under the microscope and found several ova of the same shape color and dimensions as those he had found in Tso Tong s case The organism was evidently a distome but

not feeling quite sure if it was a new species he sent it to Spencer Cobbold who pronounced it a new trematode and named it *Distoma ringeri* At about this time (1880) Baelz had found trematode eggs in the sputum of hemoptysic patients in Japan and in 1883 he recovered the adult trematodes from the lungs and named them *Distoma pulmonale* (see letter from Baelz cited below) At first there was some confusion as Baelz mistook the eggs for psorosperm cysts and held that they represented a stage in the life history of gregarines therefore he named the disease Gregarinasis pulmonum but Leuckart in Leipzig to whom the specimens were submitted recognized them as eggs of a distome

In 1892 Yamagawa and other Japanese workers found these mature flukes in atypical foci in the body Finally the life history of this *Paragonimus* which involved melaniid snails and freshwater crustacea was elucidated by Nakagawa (1917)* as well as by Mujairi Yoshida Ando Yokogawa and Kobayashi

Transcript of letter from Professor E Baelz Tokyo February 19 1881 to Patrick Manson

First of all I have to apologize for not having written to you earlier I always waited for a good case of the *Haemoptoe parasitica* to send you a specimen. At last I have got a prominent one and trust that you will be able to compare the eggs in the sputa with those which you found in the lung of a patient affected with distoma

It is not at all improbable that you will find them identical I called them gregarina because they are exactly like what is described as such but when I now send my first notice about them to Europe I saw more clearly which seemed to me to prove that the oval bodies must be the eggs of some worm and if so there could hardly be question of another than distoma I wrote this to Leuckart, and he too is of the same opinion

Therefore I am much interested in hearing that the same disease occurs in China As soon as I have published the details of my investigations I will forward you a copy I have always followed with the greatest interest, your publications in the Maritime Customs Reports especially those about Filaria I have found chyluria with Japanese 7 times in five years which is as far as I know not known to foreign physicians Tokio has about the same average temperature as the middle or south of France and as the occurrence of the disease in Japan would show it is not limited to the tropics Mosquitoes we have here of course so you will not be surpri ed hearing that chyluria might be met with in Tokio

I am dear Sir
Yours sincerely
E. Baelz

On April 24 1880 a Chinese—Tso Tong a petty mandarin—came to consult him about an eruption on his face and legs Manson noticed that

* Nakagawa K (1917) Contribution to the Life History of *Paragonimus westermani* China Med J 31 (1) 65 67

his voice was rough and loud and that he frequently hawked up and expectorated small quantities of reddish sputum Subsequently Manson frequently referred to this episode as the Tale of a Spitoon (At that time this vulgar utensil now a relic of the Victorian age was in common use Lady Manson was wont to relate how on her arrival in Amoy in 1876 she found her drawing room floor covered with sawdust and accommodating seventeen spitoons Guests who did not exhibit due skill in operating these utensils were not invited a second time)

At this time Manson was still interested in filariasis and had arrived at the belief that after their arrest in the lungs during the daytime it was reasonable to suppose that they might appear in the sputum

The patient Tso Tong tried to spit into a spitoon but missed Manson was about to rebuke him but when he realized that the sputum was mingled with blood his anger and disgust evaporated So he asked him to spit again and transferring a small portion to his microscope he beheld the eggs of a hitherto unknown worm The eggs were oval in form one end of the oval being cut off and shut in by an operculum They were granular on the surface blood stained and measured on an average 1/300 × 1/500 inch When the covering glass was pressed the egg ruptured and the granular contents escaped The shell was then seen to be brownish red in color Manson asked Tso Tong to return 2 days later and made him spit into some jars containing water he found the sputum as full of eggs as on the first occasion From July 14 to July 31 1880 this man was engaged by Manson as his house coolie so that he was assured of an abundant supply of sputum for experimental purposes Later he found another patient—*Heng*

The story continues as Manson used to relate it and tell how he came to discern the future destiny of the distome ova The jars containing the sputum were put away in his laboratory or muck room as he called it and the sediment was at first examined daily He found that the eggs hatched and gave birth to miracidia only when carefully washed free of mucus and blood and kept as long as 7 weeks

It was lucky for Manson that for at least four of these weeks he forgot all about his experiments At first the eggs refused to hatch although he could plainly discern the enclosed embryo At this point he ceased his observations till his attention was drawn some 14 days later by his wife to a curious and most disagreeable odor emanating from his laboratory the odor was found to proceed from the jars in which the mandarin s sputum was decomposing and growing fungi of all descriptions Manson resisted the pleadings of his wife to throw the nauseous mess away but characteristically produced a pipette and sucking up some sediment was soon able

to demonstrate the miracidia escaping by way of the opercula from the ova These various stages were depicted in the paper he wrote on this subject in the Maritime Customs Report (1881 China Customs Reports XX p 10 1882 XXII p 55)

It has become quite obvious that the hand of fate was the guiding principle in this affair And it demonstrated the fact—now generally known—to wit that the eggs of *Paragonimus do not hatch immediately upon coming* into contact with water but have to mature for 5 or 6 weeks before doing so But there was also a hitherto unrecorded sequel to this story that shows the direction in which Manson s mind was working He had observed the apparently purposeful gyrations of the escaping miracidia swimming around in the water as if searching for some object

This must have suggested to his mind the possibility of an intermediary host in the shape of some species of freshwater mollusk How else explain the accompanying letter found in Manson s Diary which is quoted below The full significance of this episode can be appreciated when it is remembered that Leuckart published the life history of *Fasciola hepatica* in the snail *Limnaea pereger* in 1882 which was confirmed by A P W Thomas (1883) in the following year *

The inferences that Manson drew from this experience are recorded as follows (1) In the stagnant stinking water from which the mucus had not been removed from the ova they rot and decompose (2) During the cold weather (under 70 F) development is suspended (3) The intermediary host of *Distoma ringeri* is not active in cold weather

In Manson s opinion the intermediary host was limited to a comparatively small group of animals It must be an inhabitant of fresh water common to Japan and Formosa (where endemic haemoptysis is endemic) It did not inhabit or was rare on the mainland of China or at least that part near Amoy This latter circumstance had precluded him from pursuing his investigations any further but he suggested that it should be taken up at this point by others residing in Formosa or Japan who being in the midst of the disease would enjoy ample opportunities

Hungerford s letter from Hong Kong

Hong Kong 21 X 81

My dear Manson

Your discoveries about the lung fluke are decisively interesting and any help I can give you in the matter is very much at your service

* Leuckart R Zur Entwickelungsgeschichte des Leberegels *Arch Naturgeschichte* pp 80 119 (1882)

Thomas A P W The Life History of the Liver Fluke (*Fasciola hepatica*) *Quart J Microscop Sci* 23 99 113 (1883)

I know of one fresh water species which I think answers all your requirements and of 16 shells of which I am sending you from my collection by this mail

Let me know if you would like to have a few specimens in spirit and I will write to a friend at Nagasaki to send them down The shell I speak of is *Melania libertina* (Gould) I found it at Icon lik above Quatatici and in all the streams between Jamsin and Tikchann Also it occurs in Japan at Nagasaki Hakom Manoshita and Nikko—places very widely apart so that its distribution is very extensive I have found none of this species in China however though other shells of the same genus are abundant there *Paludina chinensis* (Gray) is another shell I found in Formosa and not, I think in Amoy Still it is probably there as it is abundant about Canton and Macao and is largely eaten by the Chinese

I found a very closely allied species in the paddy field at Kulangsu much nearer to it than any of the Chinese melania which I have seen are to *M libertina* *Limnaea plicatula* (Benson) is abundant both in Amoy and Formosa so it may be put out of reckoning and the small *Stenothyra* which we had under your microscope seems to be identical with the specie *glabrata* (A Adams) I found at Amoy and Swatow and which reaches as far as Singapore On the whole I think *M libertina* must be your friend he is a hardy beast and will reach you from Tamsui alive You might send to Swatow also and see if it reaches so far south If so and that the fluke s not common the e t will tell against the theory It will be something in favour of my hobby if it helps to clear up a doubtful point in the natural history of flukes

Yours very truly
R Hungerford

It was at this point that Manson began to speculate upon the possible mode of transmission by freshwater snails and there are records in his Diary in which he discusses the possibility of contracting the infection by *eating* these mollusks

In the case of Kau a Chinese preacher from Tamsui Formosa who had been connected with the Reverend F L Mackay for nine years whom he examined for cough and hemoptysis and in whom he recorded physical signs in the right lung diagnosed as distomiasis Manson ascertained that earlier in the year (1883) Kau had eaten mollusks but always cooked and it was after this that his cough began The snails came from a freshwater river and Kau volunteered the information that one of his neighbors had done the same after which he began to spit blood profusely This note is dated November 23 1883

The second case was a cook Hai 26 years old hailing also from Tamsui He began to show symptoms at 16 years being afflicted with a cough and the production of much mucus From that time till the date of examination (December 1 1883) he had had frequent hemoptyses He had often drunk *water from a well and had also eaten snails—Sio tchin lee* Formerly this mollusk was much eaten in Tamsui but a certain man said that they eat dead man s flesh so now they are never eaten Hai volunteered that he

knew of more than ten people in Tamsui who like himself spat blood

On January 23 1884 Manson examined sputa which had been incubating in water that had been changed four or five times since November 30 1883 No advance in development was seen in the numerous ova discovered

On March 23 1884 he examined all the bottles Three stank abominably and in none of these did the eggs appear to be alive but in the two that did not stink the water was clear and at the bottom there was a thin caked layer containing many ova In one 75% were empty with the lids off in the other the ova had not undergone any change The water in all the bottles was then changed On May 5 1884 Manson went over the six bottles The ova in the stinking water had undergone no advance in development although some of them contained yolk spheres with much granular activity The others all contained ova in which the embryo (miracidium) had escaped or were well advanced in development The last note on May 26 1884 records that most embryos had escaped and the shells were empty

The first case record of endemic hemoptysis was on April 24 1880

Haemoptysis O a of Ento oa in Sputum

Tso-Tong m 35 native of Foochow resident in Amoy about one year this time Secretary to the salt office

Born in Foochow city and lived there till he was 21 years old when he went to Formosa where he resided for 4 years

A year after his arrival in Tecktcham Formosa when he was 22 years old he first spat blood every day half an ounce to one ounce and this continued for 19 days Very little cough though he was emaciated slightly Haemoptysis returned 6 months afterwards smaller in quantity and like the former pure blood unmixed with mucus Since then he has spat blood for a few days at a time every two or three months without much cough Once for two years he had no blood spitting In good general health though not very stout. Is thin naturally Has never had ague or fever never typhoid or other serious disease

Came to consult me about eczematous eruption he has on both cheeks and chin also on both legs I observed as he was speaking to me that he hawked up a small quantity of reddish sputum and that his voice was rather rough and loud. I examined the sputa which in part was made up of pellets of rusty pneumonia like sputa specks of clear red blood and ordinary tenacious mucus Under the microscope besides ordinary blood and mucous corpuscles were many oval ova——dark red blood stained measuring 1/330 × 1/100 inch granular on the surface having a lid on flattened open end at one long diameter Crushing these causes the shell to crack and granular and oily matter to escape Contents are very indistinctly marked but one distinctly differentiated embryo is visible The ova are very numerous sometimes as many as three or four in one field at a time Ova delicate double outline Shell pale brownish red Although there is no auscultatory or other sign of phthisis I cannot but think the parent of the ova and the haemoptysis are associated as cause and effect

Note This case is quoted in The Filaria sanguinis hominis by Manson (1883) pages 136-137

References on Distoma ringeri

(1880) China Imperial Maritime Customs II Special Series No 2 Medical Reports for the Half year ended 30th September 1880 Issue XX *Distoma ringeri* With 2 Plates pp 10 12

(1881) *Ibid* II Special Series No 2 Medical Reports for the Half year ended 30th September 1881 Issue XXII *Distoma ringeri* and Parasitical Haemoptysis With 2 Plates pp 55 62

XX Manson s Work in China on Other Helminthic Parasites

A *Ligula (Sparganum) mansoni*

On September 2 1881 Manson recorded the discovery in his diary

The case is headed *Lympho Elephantoid—Profuse discharge of Lymph Great debility Filariae in lymph and blood*

Operation—death Post mortem examination dysentery ulceration Tchkai Male 34 Cotton carder

Illness began at age 26 with inflammation and abscess of scrotum Six years previously discharge of lymph had commenced Then followed a series of ague attacks with cough and anorexia The scrotum itself was larger than a man s head and the penis was buried in it The femoral glands were large and varicose The greater part of the mass looked like ele phantiasis but on the left side there was a patch of large and tense vesicles from which the lymph was constantly escaping spouting in a fine stream and it was recorded that several pounds weight escaped looking like milk and containing filariae Filariae were also found in the blood On September 3 the tumor weighing over 3 pounds was removed

The patient died on September 21 from dysenteric diarrhea The vomit ing and difficulty in swallowing suggested a stricture of the esophagus Manson related in his Diary that with the aid of a trusty porter he dis interred the body in the dead of night aided by a flickering candle At postmortem the whole of the large intestine from the ileocaecal valve to the anus was found to be covered with ulcerations A number of parasites (about twelve) were found in the subperitoneal areolar tissue in the iliac fossae and behind the kidney a similar parasite was found in the pleural cavity Some were coiled up in a knob others lay extended about 12 inche long by 3/16 inch broad moving languidly like tapeworms Two hundred were in the stomach and a dozen in the ileum

The chest was normal except that in the esophagus where the left bronchus passes in front of it there was ragged ulceration and thickening

The esophagus was firmly attached to the bronchus and at the site of ulceration was so narrowed that the little finger would not traverse the stricture

The lymphatic glands of the groin over the saphenous opening were appreciably enlarged firm but not hard giving the idea that the outer part of the gland had been distended but had now collapsed The lumbar glands were enlarged In blood from the lungs and spleen there were few filariae but considerable numbers were found in lymph from the left groin

The parasites were long tapelike animals about 12–14 inches long by ⅛ inch broad and 1/64 inch thick They were dead white and moved distinctly when taken out of the body Some were lying stretched out others were massed in an irregular coil The two extremities were rounded off and rather thicker than the rest of the body A hurried glance with the microscope showed one extremity to be lipped At first placed in a mixture of serum and urine they were later dipped in spirits On examination the *whole body seemed to be stuffed with clear round ova many of which had* double or treble outlines with an appearance of a nucleus All apparently were lodged in a sort of loose fibrous matrix which split up longitudinally *or ruptured transversely The integument was very thin One extremity* appeared to have a slit in it the other was lipped but the organisms became so soft that any internal structure they might possess could not be distinguished

The life history of this parasite has now been worked out As Manson suggested his Ligula is the larval form (or plerocercoid) of a cestode worm of which the adult stage is *Diphyllobothrium mansoni* (Cobbold 1882)

It is now found in Japan China East Africa Australia and British Guiana The adult form is found in the dog wolf fox cat leopard and tiger Its plerocercoid form occurs in man frogs and snakes The adult stage occurs in the dog and other animals and the plerocercoid under natural conditions is found in a frog (*Rana nigromaculata*) or a snake (*Elaphe climacophora*) The procercoid stage develops in *Cyclops leuckarti*

B *Oxyspirura mansoni*

Oxyspirura mansoni (Cobbold 1879) Ransom 1904 syn *Filaria mansoni* Cobbold 1879

Oxyspirura parvorum Sweet 1910 according to Baylis 1934 Yeh 1957

On April 10 1878 Cobbold received from Manson a letter from Amoy announcing his recent acquaintance with a filaria infesting the eye of the domestic fowl *in Amoy Manson had removed one successfuly* from a bird in his neighbor s hen run On May 9 1878 Cobbold received a pack

age from Amoy containing the head of one such bird which showed examples of the new worm in the eye The male was 5/8 inch and the female 3/4 inch in length

Cobbold at that time proposed to call the new species *Filaria mansoni*

Original references

Cobbold T S (1879) Parasites A Treatise on the Entozoa of Man and Animals Including Some Accounts of the Ectozoa 508 pp 85 figs London. (Pp 440-441 deal with *F laria mansoni* in *Gallus gallus* Amoy)

Cobbold T S (1880) Prefatory note to Further observations on microf lariae with a description of a new species by P Manson *J Quekett Microscop Club* (44) 6 130 132

Manson, P (1880) Further observations on microfilariae with description of new species Communicated (with a prefatory note) by the President (Thomas Spencer Cobbold June 25) *Ib d* Pls 8 10 (Note by the President pp 139 140)

General description A general description and history of the worm was published in Bird Parasites of the Nematode suborders Stongylata A caridata and Spirurata by E B Cram [*Smithsonian Inst U S Natl Museum Bull* (1927) **140** 465 pp (pp 352 328 deal with *Oxyspirura mansoni*)]

Cram also reviewed genus *Oxyspirura* in 1937 (A review of the genus *Oxyspirura* with a morphological study of *O petrowi* Skrjabin 1929 recently discovered in galliform birds of northern U S *Skrjabin Commem Vol* pp 512 541)

C *Filaria papillosa*

Filaria papillosa Rudolphi 1802 syn *Setaria equina* (Abildgaard 1789) Railliet and Henry 1911

Filaria papillosa or *Setaria equina* a common parasite of equines lives mainly in the peritoneal cavity but occasionally juvenile worms may be found in the eyes It is almost worldwide in distribution but its life cycle is unknown probably it is conveyed by a mosquito

J W Thwaite reviewed the genus *Setaria* in 1927 (The genus Setaria *Ann Trop Med and Parasitol* **21** 427-466)

Manson writing to Cobbold [published in 1879 (*Linnean Soc Zool* **14** (75) 304 311] said

Some time ago I operated o i an Australian horse for this worm and had the satisfact on of find ng the parasite not very much injured after removal it was an unpregnated female possessing all the features of the Filariae

Its head was armed with a five or six toothed saw the teeth ar anged like some old fashio ed treph ne in a circle round the mouth. I removed a worm from the same eye of the same horse three or four weeks previously

I have inferred from the perfect boring apparatus and from the f male bei g

unpregnated that the eye is not the breeding or resting place of the Filaria found in it but that it is sometimes accidently entered by the worm on its travels in search of a suitable spot From the fact that one worm succeeds another I infer that the sexes are brought together in this way when a waiting worm comes across the track of another it follows it up Thus several may be found together at the end of the burrow

I cannot say if the three or four papillae round one extremity constitute the perfect boring apparatus of the worm or if it is a boring apparatus at all but comparing it with what is found in other species of the same genus I think it is very probable that it is either in or will become the piercing apparatus

[See also Cobbold s (1879) A Treatise on the Entozoa of Man and Animals (p 383) listed in Section XX B]

D *Trichina spiralis*

On account of the number of pigs in Amoy and from the fact that pork constituted one of the main dishes of the local Chinese Manson became suspicious that many of the severe cases of illness he saw might not be due to this infection Accordingly from April 1 to May 8 1881 he examined 219 specimens of home grown pork for trichinae and found only two in fected—one on each of two occasions

The first trichina was found in a series of seven specimens of pork A dog (or puppy) a few weeks old was fed about a square inch of this meat It became very mangy and lazy and thin bellied When it was killed on June 6 1881 its muscles were found to be full of trichinae some encysted and others still free and active

XXI Conclusions

It may be that this paper may be regarded as a highly colored eulogy of Patrick Manson but it has not sufficed in this critical analysis to over emphasize the occasions when he was right—it has always been my aim to point out where he was subsequently proved to be wrong This is quite natural because to be proved right in every detail in a work of this mag nitude one must be almost superhuman

The task to which I have set my hand is at its close and it is hoped that this account of Manson s pioneer work in China may prove to be of some historic value as demonstrating perhaps more clearly than heretofore how this man of genius urged by the cold logic of his observations was enabled first to postulate and later to prove that his filaria underwent a meta morphosis in the body of a particular mosquito in order to perpetuate its species

By so doing he was the first to prove beyond cavil the mechanism of insect borne disease Thus he had demonstrated a great and new principle

which since has stood out as a landmark of medical science—one which has led to the transformation of the Tropics thereby conferring one of the greatest benefits on mankind The boon that it has been to the world at large transcends the imagination The revolution in medical thought that it brought about can fairly be compared to the impact on the physical sciences by the discovery of atomic energy and may have been largely responsible for the recently computed addition of forty million inhabitants of the world in one year

An Outline of Xerophthalmia

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> Never while memory lasts can one obliterate the mental picture of those pitiful little bundles of marasmic apathetic humanity lying in the arms of gaunt women their faint fretful wails ring still in one s ears to-day summoning up visions of wasted stick like limbs of distended abdomens of dry inelastic scurfy scaly skins of hair scanty brittle and dry and of sightless desiccated eyes
>
> R. H Elliot (1920)

I Introduction

Between the incidence in many parts of the world of xerophthalmia and the number of physicians an inverse relation often exists The ultimate cause of xerophthalmia is so clear the clinical picture so typical and the prevention so easy that there would be no excuse nowadays for a large scale occurrence of the condition in adequately staffed well-off temperate regions The appearance of an endemic as occurred in Denmark forty years ago would be inconceivable today except under very abnormal conditions

Less assuring is the situation in some regions of the Tropics even where a lush vegetation indicates an abundance of carotene There this dreadful affection is sometimes a widespread scourge with an ominous predilection for small children It is rarely a man made disease such as beriberi which ultimately may be ascribed to a taste for refinement or to the dawn of the industrial age The prevalence of xerophthalmia has been diminished by increasing understanding of the need for specific nutrients but—where it is most urgent—far less so than in beriberi In this epoch the affection as a health problem seems to favor the Tropics Not in the sense that either climate or temperature influence it but in that it is closely linked with low economic status To an ideology that aims at the improvement of health for every human being xerophthalmia is still a problem of importance But even if there are aspects requiring further elucidation there should not be deficient knowledge or ability on the part of the physician Excellent reviews have appeared on the subject by Pillat in 1939 and by Bietti in 1940 Yet neither seems to have drawn the attention which the subject deserves Since then new developments have taken place one of the most important being the increasing interest in infantile malnutrition

In this review the emphasis will be on endemic xerophthalmia Blumenthal (1950) called malnutritional keratoconjunctivitis a sociological disease in South Africa very much the same could be said of xerophthalmia as regards southeast Asia

For a proper understanding it should be mentioned that the author s personal viewpoints are based on a long and intimate experience with the condition in Indonesia

II The Idea

Early History of Xerophthalmia

With few exceptions most of the papers on xerophthalmia in the nineteenth century represent clinical casuistry Von Graefe (1866) in Berlin often saw three or four cases per month Institutions for children as a source of origin repeatedly appear in the reports since Bitot (1863) described his spots in twenty nine debilitated French orphans The first mention from the Tropics was that of Gama Lobo (1865) from closed communities of Negro slaves on Brazilian coffee plantations But it took some time before another tropical country—Indonesia—came into the picture (Ouwehand 1900)

In many cases xerophthalmia was associated with other affections As early as in 1843 a paper by Fischer (1843) describes it in the wake of measles Teuscher (1867) de Gouvea (1883) Weeks (1887) drew attention to the frequent combination with intestinal disturbances Of course often the etiology interested the various authors The superficial affection had to be differentiated from interstitial or parenchymatous keratitis by other causes A relationship to poor general health has been apparent from the first studies It has been related to vague cerebral syndromes called infantile encephalitis (von Graefe 1866 Hirschb rg 1868 Jacusiel 1875) which afterward appeared to be incorrect In the period of triumphs of bacteriology a microbial agent has often been discussed (xerosis bacilli by Franke 1886 Gallenga 1888 Schreiber 1888 a capsulated bacillus by Loeb 1891 molds by Leber 1883) However though admittedly the xerosis bacillus plays a role it was soon reduced to a secondary phenomenon (Bietti 1903) Quite different were the aspects relating xerophthalmia to liver disease by the coming of the term *ophthalmia hepatica* by Baas (1894) In his case two different conditions of the eye the superficial affection and an alteration of the retina were connected with liver disease though by different pathways (Purtscher 1900) Though liver disease often was related to xerophthalmia afterward Baas s term soon became controversial and obsolete A similar secondary relation probably existed in cases connected with abuse of opium (Paster 1886) or of alcohol (Bernhard 1902 Kreiker 1930)

In the older reports infants represent a large proportion They often suffered from a social or congenital vulnerability such as neglect especially as to nutrition or debility in prematures twins etc Some authors considered the affection as specific for infants (Weeks 1887 Braunschweig 1890 Krausse 1899)

During the nineteenth century a definite connection between diet and xerophthalmia had haltingly been established and the epidemiological character was not clearly recognized

Dujardin (1895) in France and Basso (1897) in Italy were probably the first to draw attention to the latter aspect and both the dietary causation and the periodical accumulation of cases were stressed in the extensive reports of Mori (1904) from Japan

Later these pecularities of xerophthalmia received much attention In spring the number of cases was reported to increase in Germany (von Hippel 1913 Grossmann 1913) and in Russia this increase was explained by the Lenten fast (Hamburger 1923) Mori had already stressed the shortage of fat in the diets of his patients and it was observed by Czerny and Keller (1906) and Roenne (1916) that the condition appeared simultaneously with the syndrome ascribed to a predominance of carbohydrate in the diets of infants (*Mehlnahrschaden*)

The increasing interest and knowledge and the evident frequency of the affection are reflected for instance by the seventy papers which Bietti (1940) encountered in many European and in one Japanese medical journal between 1875 and 1900

It may be of interest to note that thus nearly all clinical characteristics of xerophthalmia were understood before the causation by the fat soluble factor was realized The affection of growth and general health the promoting effects of disease and debility the predilection for young children the epidemiological character in the absence of a contagious agent the deficiency of fat the curative properties of cod liver oil and the preponderance of carbohydrates in the diets and the simultaneous presence of hemeralopia constituted indeed a syndrome *sui generis* Some of these facts have sometimes been obscured in later decades by the emphasis on a single dietary factor and the single affection of certain tissues of the eye

The further development of our knowledge need hardly be emphasized especially regarding the causative connection with vitamin A and carotene or the linkage of xerophthalmia to hemeralopia These facts are well known and well publicized for instance in the recent monograph on vitamin A by Moore (1957) This however does not mean that adequate attention has since been paid to the easily preventable and eminently curable disease itself Apart from the dominating position of the vitamin in the etiology there are other alimentary factors involved Because of

their practical importance they will be reviewed later on together with the interference by infectious disease

III The Name

A Etiological Terms

As regards nomenclature not only terms to designate nosological or pathological entities demand attention we cannot avoid pondering etiolog ical terms as well Xerophthalmia as described here is probably always a manifestation of avitaminosis or hypovitaminosis A This refers to the impoverished tissues not to the alimentary intake The variety in which the oral intake is reasonable but where metabolic causes (liver and pancreas disease etc) prevent a normal utilization has rightly been called dysvitaminosis (Collevati 1931) This term may roughly indicate the problem in situations where the alimentary intake of preformed vitamin (axerophtol) is adequate But two thirds of mankind after the breast feeding period has to rely on vegetable carotene instead and the resorption and conversion of this substance may be defective In such a case the term apraxia for the provitamin is appropriate (Tijssen 1940 Oomen 1958) Carotene is not a vitamin extended though the term sounds now adays and the units of potential activity are correct only insofar as a quantity of fuel is an indication for milage For practical purposes hypo vitaminosis A is an apt enough term provided that the condition is not considered only from the angle of an alimentary deficiency

B Ophthalmological Terms

The description of symptoms of xerophthalmia in Section IV indicates that there are differences and shades in the manifestations of the syndrome There is little sense in denying the essential similarities in the eye symptoms in man and in some experimental animals But we should be well aware that even minor deviations in anatomy and physiology may ultimately have profound effects on symptoms and therefore on termi nology Of such several may be enumerated In no mammal is such a large area of the bulbar conjunctiva exposed as in man Rodent eyes are round and bulbous with lids closely fitting around the cornea Rodents have Harderian glands whereas man has not The Meibomian and the lachrymal glands in man are large The metabolism of carotene and the relative liver reserves of vitamin A show distinctive differences even in closely related species The edema of the eyelids the abundance and the nature of the abnormal secretion and the degree of inflammation in ex perimental animals are not congruent with the affection in man

If it is relevant even to consider differences in age sex and even race

(McLaren 1956) in the phenomenology of the disease we should be very careful with the implications of terms indifferently used for man and for animals A case in question is that for the condition experimentally caused in rats McCollum and Simmonds (1917) used the term xerophthalmia Osborne and Mendel (1921) ophthalmia and Goldschmidt (1915) and Stephenson and Clark (1920) keratomalacia

In fact as regards terms not much progress seems to have been made since Hoor (1906) before the discovery of the vitamin discussed the nomenclature He pointed out the essential difference between external and internal causes of xerosis and it is still worth realizing that both overlap now and then He mentioned a xerotic keratitis related to a paralytic keratitis or a keratitis *e lagophthalmo* in semicomatose children *with eyes half open and suffering from acute diarrheal diseases* On the other hand he insisted that a fatal diarrhea sometimes occurred in children with keratomalacia which developed inside closed eyes A child with true keratomalacia is often cachectic and a cachectic child sometimes develops a xerotic condition of the cornea though this need not be the variety which interests us here

There is or has been controversy about the significance of the terms xerophthalmia xerosis corneae and keratomalacia Hoor (1906) and Pillat (1939) contend that there is no use for the term xerophthalmia or xerophthalmus Owen and Hennessey (1932) advocate a term covering the diverse specific alterations yet not committed too narrowly to any single one

In references to this disease xerophthalmia is frequently used as a term synonymous with keratomalacia It would seem reasonable to draw some measure of distinction between the two terms for xerophthalmia or dryness of the eye may be confined to the conjunctiva only while keratomalacia implies corneal change The use of the term keratomalacia is strictly speaking only admissible when signs of corneal necrosis are apparent though still admissible when the earliest and minutest evidences of such necrosis are visible Xerophthalmia is therefore the wider expression and we propose to use it to include any of the external ocular manifestations of vitamin A deficiency although the term keratomalacia will be used when it is desired to stress the corneal necrosis Similarly the expression xerosis conjunctivae will be used when it is necessary to emphasize that the process is confined to the conjunctiva

There is a serious practical objection to foregoing xerophthalmia lightheartedly it is already too well established Though by no means denying that it belongs to the specific alterations of the eye caused by hypovitaminosis A we prefer to retain the term *xerophthalmia as indicative of the clinical syndrome as a whole*

It seems superfluous to discuss the term xerosis conjunctivae especially if it is mentally supplemented with epithelialis and eventually bulbaris But there are apparent difficulties in handling the terms xerosis corneae (epithelialis) and keratomalacia Terminologically a xerosis is of course not a malacia and vice versa But if defects of the cornea occur owing to local necrosis—sometimes called ulcers and soon showing a small prolapse of the iris following a perforation—should the condition then be conceived as developing from xerosis to malacia? It is clear that many authors failed to pose this question and that a serious loss of corneal surface always meant keratomalacia as in the case of the quoted authors Van Manen (1938) insists on calling keratomalacia the stage of corneal affection in xerophthalmia There is no reason at all to use the adjunct kerato if the cornea is not (yet) affected Because of the vari ty in corneal affections related to xerophthalmia it is pleaded here that keratomalacia should be reserved for the *most destructive* stage

Authors like Ruete (1845) speaking of emollities corneae and von Graefe (1866) of Verschwarung were concerned with the sudden destruction of the whole of the cornea described by Kirkpatrick (1922) The tissues of the cornea melt away like thawing snow the entire structure yields en masse to the intraocular pressure the lens is extruded while phtisis bulbi is the final result

Reviewing the many terms that are or have been used to designate the affection (Table I) it is interesting to note that apparently controversial names indicate stages of the—basically—same condition A xerosis corneae cannot be reconciled with an emollities corneae (Ruete 1845) or with an acute ulcerative softening (Rosmini 1872) or a liquefaction (Kuthe 1898) not to speak of a malacia

Though entirely open to the argument that there may be racial differences and that the picture differs according to the age of the observed patients we propose to refer in the clinical description to a softening of the whole cornea as keratomalacia while distinguishing it from localized partial possibly multiple epithelial defects so often present in the wake of a true xerosis corneae

C General Terms

Another feature derived from the consideration of older and newer terms should be noted The nomenclature of the disease often contains elements that indicate an affection of a general nature (cachexia dystrophia) or of a very different organ (ophthalmia hepatica) or that refer to a nutrient which is not vitamin A (lipaporia dystrophia alipogenetica) This we have to remember when discussing the broader consequences of hypovitaminosis A or the pathogenesis of symptoms

In passing it may be remarked that most of the terms mentioned were often pronounced in the same breath with hemeralopia However the close connection between these two very different conditions will not be discussed here extensively

TABLE I
SOME TERMS USED TO CHARACTERIZE THE SYNDROME OR THE STAGES OF XEROPHTHALMIA

Author	Year	Term
Mackenzie	1830	Conjunctivitis arida
von Ammon	1830	Xerosis conjunctivae
Ruete	1845	Emollitis corneae
Bitot	1863	Bitot s syndrome
Gama Lobo	1865	Ophthalmia *brasiliana*
Hirschberg	1868	Ulceration of the cornea
Rosmini	1872	Acute ulcerative softening of the cornea
Kuschbert and Neisser	1883	Xerosis epithelialis conjunctivae
Leber	1883	*Infantile ulceration of the cornea*
de Gouvea	1883	Xerophthalmia *cachectica*
Borthen	1886	Ophthalmomalacia
Schoeler	1887	Xerotic corneal disease
Biber	1890	Keratomalacia *infantum*
Baas	1894	Ophthalmia *hepatica*
Kuthe	1898	Liquefaction of the cornea
Nesnamow	1900	Nonulcerative progressive turbidity of the cornea
Mori	1904	*hikan* xerosis conjunctivae et corneae *infantum lipaporia*
Uhthoff	1904	Keratitis xerotica
Hoor	1906	Xerosis conjunctivae et corneae epithelialis keratomalacia
Attias	1911	Keratitis exfoliativa
Bloch	1919	*Dystrophia alipogenetica*
Ross	1921	Nutritional keratomalacia
Bondi	1922	Xerosis hemeralopica
Blegvad	1924	*Dystrophia* xerophthalmica
Narog	1928	Xerosis epithelialis simplex xerosis diffusa degenerativa
Pillat	1939	*Xerosis epithelialis conjunctivae et corneae* keratomalacia (mummification prexerosis)

a Terms indicating more than an eye affection in italics

Though the clinical picture of xerophthalmia as a whole is typical enough there are small differences between children and adults and between the white and pigmented races As the young child is probably most frequently the sufferer we prefer for a standard description the condition as observed in the cafe au lait-colored Indonesian toddler

IV The Eye

It is difficult to describe xerophthalmia in man in concise terms It has been attempted in numerous textbooks but except in the more specialized treatises a realistic picture is rarely evoked

Wright (1929) characterized the disorder as follows The lachrymal glands cease to produce tears the outer layers of the cornea undergo necrosis inflammatory processes occur in the conjunctiva and may involve the anterior and posterior chambers of the eye leading to complete blindness

Moore (1957) still referring to Bloch s cases delineated it as Xerosis or drying of the conjunctivae was the first visible stage Later this xerosis extended over the cornea which first shrivelled up and then developed regions of necrosis with ulceration and often perforation The stage known as keratomalacia had then been reached

Neither is a recent formulation of Banerji (1958) ufficiently to the point The earliest noticeable change is decreased lachrymation The conjunctivitis is a dry conjunctivis accompanied by itching and burning and redness of the conjunctiva Follicular conjunctivis and granular lids have been reported as a result of the deficiency and this stage has been called prexerosis Another early change is cornification of the epithelium of the cornea and scleras On the scleras a piling up of epithelium forms localised spots areas of thickening with decreased tran lucence

If uncomplicated no stage of xerophthalmia is an inflammation Red ness and burning sensations are not typical manifestations Prexerosis is a functional lesion occurring before visible changes of the tissues are apparent The sclera is affected only in extreme cases of malacia Though the conjunctiva is thickened there is no que tion of a piling up of cells

If we must delineate the affection in a few lines we would prefer the following *definition*

Xerophthalmia is a dyskeratosis of the clear exposed epithelia of con junctiva and cornea The conjunctiva shows xerosis a typical dryness or unwettability by tears combined with increasing opacity thickening of folds and abnormal fatty secretion collecting outside the limbus as Bitot spots on the interpalpebral conjunctiva In colored races a transient hyper pigmentation occurs In a later stage the cornea is affected first super ficially by dryness roughness and anesthe ia But soon small localized deeper defects appear causing prolapses of the Descemet membrane after perforation showing a tiny iris slip In the severe stage called kerato malacia mostly in children the cornea as a whole is affected and suddenly melts away Diverse types of infection develop but are secondary in

nature Blindness is frequently a sequel The eye affection constitutes only part of a syndrome resembling protein malnutrition

The frequent connection of xerophthalmia with hemeralopia suggests a relation with changes in the fundus oculi They often may be functional only but visual and anatomical alterations have been described in individual cases The close relation with our subject has been stressed recently by the report in 54 cases of hemeralopia and xerosis from Java of typical granules scattered over the periphery of the fundus (Teng Khoen Hing 1959)

A XEROSIS EPITHELIALIS CONJUNCTIVAE

Xerosis is perhaps not the first symptom of the affection Hemeralopia—or to be accurate scotopic vision—usually precedes it In a nightblind child a slight degree of xerosis is often present and if the cooperation of a child with Bitot spots allows observation it is usually found to be nightblind

Xerosis is sometimes observed in chronic destructive conjunctivitis as in the late stages of trachoma and in pemphigus conjunctivae The ophthalmologist then uses the term xerosis bulbi and not xerophthalmia

Xerosis in xerophthalmia consists of a typical dryness and opacity of the bulbar conjunctiva preferably in the eyeslit accompanied by roughness and thickening which is apparent during movements in fine or coarser folds especially in the outer canthus On inspection with the slitlamp the translucent conjunctiva which normally looks clear like an aquarium crossed by blood vessels appears to be milky owing to fine droplets Soon the vascular pattern apart from the larger arterioles becomes obscured (Fig 2) The hue then is dirty white in some cases clay colored or muddy In the space of Bitot that is at the foot of the slope of the slightly bulging cornea in the eyeslit an abnormal sticky secretion accumulates in a more or less triangular shape The alteration of the substrate is important The earliest sign according to Jelliffe (1955) in Indian children is sometimes a *black* spot This is due to smearing the eyelids with kajal a mixture of carbon and grease which collects in the same space The *Bitot spot* looks like a plaque or a pseudomembrane and on closer inspection appears *to be riddled with fine air bubbles (Figs 1 2 and 4) Bitot compared it* with a deposit of half congealed foam (Bitot 1863) Sometimes the surface is not solid but drawn out in fine frothy stripes (Figs 1 and 2) Another time the substance looks like greasy paint without air bubbles (Fig 3) Of these signs *Nicholls and Nimalasuriya (1939) stated* It may be that there are two forms of xerosis of the bulbar conjunctiva an acute form in which the changes take place rapidly forming loose

accumulations of degenerate epithelium which appear foamy and a more chronic form in which the accumulations of epithelium take place more slowly and are more compact

The consistency varies It may be difficult to scrape off or in another case it may be a sticky fluid Between the fingers it feels greasy (de Gouvea 1883) A smear under the microscope is seen to abound in xerosis bacilli cellular debris and fat droplets On some xerotic eyes no spots are visible but small milky flakes of inspissated mucus swimming in the conjunctival pool prove that the abnormal secretion exists (Fig 3) In such eyes provided there is no serious corneal defect vascular injection is not a characteristic However irritation or a minor inflammation may emphasize the factor redness in the picture

On inserting a speculum which is not too difficult as the surfaces are anesthetic the Bitot substance coats the coarse perilimbar folds as a fatty paint (Fig 5) Especially the lateral medial and inferior parts are affected The dryness is seldom demonstrable on the palpebral conjunctiva Tears may flood the affected conjunctiva for a short time but as soon a they subside the dry spots break through the moist surface

The spots are larger on the temporal side Both eyes are usually affected but the degree may vary considerably The Bitot spot is not a type of xerosis but an accidental phenomenon Bietti (1940) suggests that the underlying conjunctiva is perfectly normal or at the most slightly dry This is not our experience The abnormal secretion gathers in the typical pattern in the Bitot space because of the relative room and the roughness of the surface (Calderaro 1899) Even if the shape reacts to lateral movements of the eye it is doubtful whether it is whipped by them into a foam as Kreiker (1930) supposed The air bubbles may be caused by the xerosis bacilli (Oomen 1958) In the course of days also if untreated the shape of the spots varies We have the impression that they are particularly large in quiet or obtuse persons The extreme case of Narog (1926) in a Polish shepherd may prove thi

Poels *et al* (1958) called cases intermediary that presented a condition which might be called prexerotic affecting mainly the interpalpebral con junctiva They were clearly related to Bitot spots The interpalpebral conjunctiva becomes more pigmented drier and more brilliant pressure on it causes a characteristic wrinkling Such symptoms may be typical of slight affections of long standing or of a very slow process of healing

The most extensive and accurate series of patients showing Bitot spots while not having signs of hemeralopia or xerophthalmia and not present ing low serum levels of vitamin A or carotene has been described by Sie Boen Lian (1938) The series of nineteen cases includes 16 Javanese an Arab a European and a Chinese They ranged in age from 11 to 44 years

1

2

and all but one were males A few suffered from trachoma The serum levels were above the average in eight cases The histological findings on *the spots in four did not significantly differ from those in genuine cases* Vitamin A therapy did not influence the affection Sie Boen Lian (1938) suggests that a considerable number of Bitot spots in adults belong to the same category Patients of Basu and De (1941) presenting Bitot spots which did not react to prolonged administration of vitamin A may belong in a similar category The high incidence of Bitot's spots observed by McLaren (1959) in Ethiopian schoolchildren not presenting low vitamin A *plasma levels and no impairment of dark adaptation may also prove* that the genuine Bitot spot is not a unique feature of xerophthalmia Despite this the spots are a very useful indicator of xerophthalmia in young children

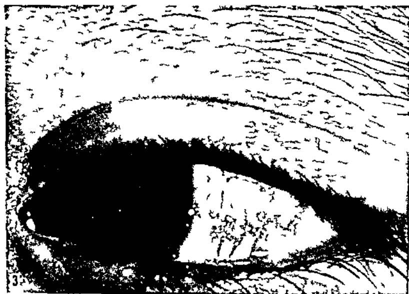


PLATE I

Xerosis epithelialis conjunctivae bulbaris

FIG 1 Male 3 years old Striated Bitot spot opacity and pigmentation of exposed bulbar conjunctiva.

FIG 2 Male 15 years old Striated Bitot spot Unexposed parts of conjunctiva are still transparent and show clear vascular pattern without pigmentation

FIG 3 Male 7 years old Flowing grease-like Bitot spot with extensive change of bulbar conjunctiva only

4

5

B Pigmentation

It is not always easy to decide whether a given pigmentation is a symptom of xerosis or whether it is a racial or genetic quality. The conjunctivae of young Javanese are pigmented even when it is reasonably certain that the children are consuming enough vitamin A and are in a fair state of health. However the specificity in a patient is difficult to judge as the specific pigment persists for months and if cured the case usually is not seen again. On the other hand it is striking that cases of xerosis and of hemeralopia of long standing always show a type of diffuse pigment in the conjunctiva which is rarely present in their healthy counterparts (Fig 7). After treatment it fades in some weeks or at most in some months. It is especially pronounced on the exposed bul

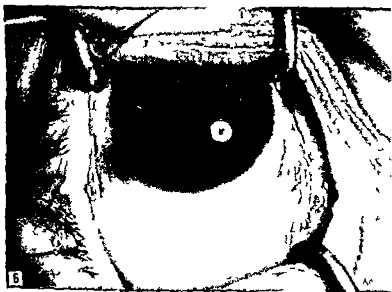


PLATE II

Xerosis epithelialis conjunctivae bulbari

Fig 4 Female 9 years old Circumscribed Bitot spot on (f al ng) tran pa conjunctiva (Patient is shown in Fig 24)

Fig 5 Male 4 years old Thickened dry conjunctival f ld with dislocated Bitot spot

Fig 6 Same eye after 5 days administration of cod liver oil per os Conjunc glossy and wet Bitot spot gone Remaining pigmentation Note appearance f ll cul

conjunctiva There is perhaps no Bitot spot which after healing is not betrayed by a distinct brown cloudy discoloration At the same time the pigment is present in the paralimbar portions of the *cornea* also when an epithelial affection is not yet demonstrable We are inclined to believe that there is a slow appearance and disappearance of a type of pigment which more or less parallels the state of hypovitaminosis Conjunctival pigmentation is not a characteristic of pure protein malnutrition

C Xerosis epithelialis corneae

Corneal xerosis is accompanied by conjunctival xerosis except when the picture is disturbed by a secondary infection or by corneal destruction (Wille 1921) It is a later and more serious stage In its early stage



PLATE III

Xerosis conjunctivae et corneae

Fig 7 Male 23 years old Hyperpigmentation and slight xerosis in a case of relapsing xerophthalmia Note enlarged pores of Meibomian glands

Fig 8 Male 3 years old Dry thick opaque hyperpigmented conjunctiva no Bitot spot Haziness of cornea manifested by blurred flash reflex (other reflexes incidental)

Fig 9 Male 2 years old Healing corneal defect with thick circumscribed halo of infiltration

it is apparent by a diffuse haziness and dryness which soon returns after a temporary wetting by tears The reflex of a light source (flash) is blurred (Fig 8) At an indefinite moment small erosions appear soon marked by a halo of infiltration Or the appearance of a hypopyon may indicate a loophole for infection A day or so later the erosion has developed into a small ulcer the intraocular pressure causes the Descemet membrane to prolapse A small black bead appears consisting of an iris slip 1–3 mm in diameter the glistening of which contrasts with the dullness of the cornea It is mostly situated in the lower half commonly in the nasal quadrant These early perforations may be irregular or multiple soon become overt and in some cases are large enough to let the lens slip through

As long as xerosis is demonstrable and the general shape of the cornea is intact we prefer to designate the stage as xerosis and not as keratomalacia The minute changes referred to by Owen and Hennessey (1932) do not yet exclude or dominate the state of xerosis As indicated earlier xerosis and malacia are more or less opposite terms In case of a single perforated ulcer due to other causes the ophthalmologist does not use the term either Even when a xerosis corneae is complicated by ulcers affecting the deeper layers it still is a xerosis

D KERATOMALACIA

Though affecting the same tissue malacia is a different process Von Graefe (1866) characterized it as a progressive ulcerous fusion in which the cornea wholly or except for a thin marginal zone is destroyed It has been distinguished from xerosis corneae by several authors (Groenow 1904 Mulock Houwer 1935) Even if the distinction was accepted the nomenclature sometimes was not followed Infantile ulceration of the cornea with xerosis of the conjunctiva is synonymous with xerophthalmia but as the most characteristic changes take place in the cornea keratomalacia is possibly the term of choice (Ross 1921) It is sometimes preceded by a recognizable conjunctival xerosis After the cornea has suffered gross damage there is not often opportunity to observe this Keratomalacia (in children) is a far more acute process lasting only some days perhaps only a single day It is a colliquative necrosis of the whole cornea sometimes apparently even affecting the limbar sclera In keratomalacia the corneal structures melt into a gelatinous mass of which the shape is so much molded by intraocular pressure that the impression of the eyeslit sometimes can be seen as a transversal ridge In case of keratomalacia stronger terms are desirable than ulceration only A striking feature of the condition is that initially it is accompanied by so little reaction of the surrounding structures

From descriptions of various origins (Japan China India Indonesia) it is clear that vast stretches of the corneal surface are immediately affected Sometimes the central beginning is stressed sometimes the peripheral crescentic or quadrantlike (Straub 1927 Pillat 1939 Bokil 1953) The destroyed corneal mass may look brownish or dirty yellow perhaps by simultaneous infiltration or now and then like a clear jelly The term perforation is often used in keratomalacia Though iris lens and vitreous often prolapse this is an understatement After malacia the cornea or the best part of it no longer exists There is apparently no demarcation as in local destruction but infection and eventually panophthalmia are always close at hand Sweet and Kang (1935) report that the epithelium first is hyperplastic and the stroma shows edema Then follow necrosis and ulceration

In contrast with some sequel of xerosis it is a diffuse process The stages of the affection indicated by Bietti (1948) to be infiltration ulceration and softening may sometimes be discernible in an adult patient but seldom in an infant which generally is the chosen victim

There are apparent similarities between a xerosis with partial destruction of the cornea and the total destruction by keratomalacia One stage may be present in the right eye and the second in the left There is however reason to distinguish because of certain consequences to the general state of the patient and because of the malnutrition keratoconjunctivitis described by Blumenthal (1950) An eye affected by keratomalacia is lost even when panophthalmia does not intervene If the child survives (outside the hospital this is very questionable) phthisis bulbi is the result If a child with destructive xerosis of the cornea survives it presents a leucoma but the shape of the bulbus remains unaltered

F OTHER EPITHELIAL MANIFESTATIONS OF XEROPHTHALMIA

Closely connected with keratomalacia but producing a very different result is *mummification* of the cornea This condition has been compared by Pillat (1929) to a dry gangrene as opposed to keratomalacia which may be considered as the wet form The whole cornea dries up into a tough leathery yellowish disk which replaces the translucent epithelium as a circular dry scab In a surviving patient the usual sequel of mummification is probably a big staphyloma

Pillat (1930) also drew attention to a conditional xerosis of the cornea which he called *prexerosis* It is characterized by a drying up of the apparently normal cornea when the eyelids are held open for 30 seconds by the presence of xerosis bacilli and by hypoesthesia of the cornea He considered it (Pillat 1939) as a link between xerosis and keratomalacia in adults Pillat's conception of keratomalacia is broader than ours an

intermediate link between xerosis perforans and malacia would consist of an extensive but not yet total destruction of the cornea But the author apparently refers to an independent condition preceding the manifest xerosis of the cornea An adolescent patient of ours for years complained intermittently of failure of vision On examination he had a hemeralopia a heavy *pigmentation of the conjunctiva and a rapidly drying* con junctiva and cornea when the eye was held open for half a minute After administration of cod liver oil for some weeks vision improved and the surfaces became bright again He then used to forget about the oil to be reminded of it again by renewed eye complaints after half a year

Prexerosis in the sense of an initial symptom before the usual anatomi cal lesions are visible is often found to be present in malnourished children but if not accompanied by hemeralopia or by visible alteration of the conjunctiva is not a convincing symptom of xerophthalmia However the incipient water repellent quality of the epithelia may help in establish ing a diagnosis

F Adjacent Structures

Though the term xerophthalmia pertains only to the lesions of the conjunctiva and the cornea there are several topographically or etiologi cally associated symptoms that are of practical importance to the clinician The function of the eye even apart from vision depends on an intricate constellation of epithelial structures partly external partly in ide the lids Various glands have to be considered (1) the lachrymal gland *inside the orbita constantly wetting the moving surfaces (2) the* Meibom glands inside the tarsus depositing a sebumlike secretion through a string of large pores on the rim of the lids (3) small numerous glands (goblet cells) scattered through the subepithelial layers of the conjunctiva producing a mucous substance Apart from the condition of the *epithelium* of conjunctiva and cornea itself and of the neural and vascular systems connected with it the function of these glands should be scrutinized when considering an affection predominantly characteristized by dryness Not so closely linked with the xerosis of the surfaces of the eyeball is the surrounding skin covering the rim of the eyeslit between the implants of the eyelashes and on the eyelids This is a fine and in its moving sections extremely functional skin It may be as good an indicator of *what happens to the skin in hypovitaminosis A as any part el ewhere*

In xerophthalmia there is often but by no means always a lack of tear secretion A young patient at examination as long as he is not emotionally disturbed has dry eye surfaces but as soon as his neurovegeta tive system is stimulated he may shed tears in abundance (Fig 20) but the tears do not wet the surface for more than an instant The latter is

nwettable Another child may cry but without tears—a phenomenon ot rare in severely malnourished children generally Likewise a disturbed hild suffering from malnutrition may not drool or sweat as does his ealthy counterpart

Von Arlt (1851) had already found the lachrymal gland to be very trophic in a case of xerophthalmia Davies (1954) considered this to e one of the specific changes of glands in protein malnutrition There ay be two reasons for a diminished flow of tear fluid in xerophthalmia One is the ab ence of stimulation through rarity of reflexes because of ypoesthesia of the cornea the second is a decrea e in function of the land caused by malnutrition As regards specificity the former may be cribed to lack of vitamin A in the corneal tissue the latter could have wo causes the keratinization of the ducts which is an effect of vitamin A eficiency and the degeneration of the secreting parenchyma which i ore typical for protein deficiency There is however no need to empha ize the lack of function of the lachrymal gland as the anatomical changes f conjunctival and corneal epithelium present a ready enough explanation f the main symptoms of xerophthalmia

Besides the decrease in irrigation by tears the lack of a bacteriolytic gent lysozyme normally present in tears has been held re ponsible for he role which infection plays in the causation of the syndrome (Findlay 925) This sub tance has been found to decrease in the tears of xerotic yes (Frank 1934) an observation which would well fit in with the eduction in enzyme secretion commonly present in protein maln trition The instillation of normal tears in affected eyes has been stated to have beneficial effect (Fuchs 1933) but Findell (1936) applying egg hite which i particularly rich in lysozyme could not ob erve any melioration in the eyes of rats nor an inhibition of the growth of xero is acilli

The role of the Meibomian glands has not frequently been discu ed En argement of the pores of the tarsal glands is a regular phenomenon perhaps n all cases of xerophthalmia of some duration (Figs 7 and 12) They ap ear like a rosary on the palpebral rim Thi is however al o present in many ca es of infantile malnutrition in which the epithelial surfaces of the eye are not visibly affected It is clearly recognizable in figures of Blu menthal (19 0) depicting malnutritional keratoconjunctivitis in a Bantu Negro Occlu ion of the pores by dyskeratinizati n would perhaps offer the e t explanation as the proces of hyperkerato is follicularis is often de mon trable in the skin urrounding the eyes and el ewhere in patients with xerophthalmia If it is not yet pre ent during the acute stage it may appear during treatment (Fig 6) In xerophthalmia patients chalazia sometimes

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The role of the Meibomian glands has not frequently been discussed Enlargement of the pores of the tarsal glands is a regular phenomenon perhaps in all cases of xerophthalmia of some duration (Figs 7 and 12) They appear like a rosary on the palpebral rim This is however also present in many cases of infantile malnutrition in which the epithelial surfaces of the eye are not visibly affected It is clearly recognizable in figures of Pluimenthal (1950) depicting malnutritional keratoconjunctivitis in a Bantu Negro Occlusion of the pores by dyskeratinization would perhaps offer the best explanation as the process of hyperkeratosis follicularis is often demonstrable in the skin surrounding the eyes and elsewhere in patients with xerophthalmia If it is not yet present during the acute stage it may appear during treatment (Fig 6) In xerophthalmia patients chalazia sometimes

occur with or without inflammation Pillat (1939) states that they have a creamlike contents and that they may appear within hours Sweet and K ang (1935) in autopsies observed metaplasia of the ducts of lachrymal and Meibomian glands The role of the secretion of the Meibom glands in the formation of Bitot spots has never been discussed but still is worth pondering

A look at the skin of the eyelids usually reveals a few peculiarities The eyelashes are often dry irregular and straight in infantile malnutrition (Oomen 1955) as also in many cases of infantile xerophthalmia As a specific symptom they may be of less significance than Jelliffe (1955) suggested The palpebral skin is often dry and lacks the mat sheen of healthy subjects the subtle difference may best be remarked after convalescence More frequently—and perhaps more closely linked to lack of vitamin A—is a fine dry eczemalike dyskeratosis between the ciliary implants Pillat (1930) also mentions a diffuse or irregular pigmentation on the eyelids but if this applies to adult Chinese it was never striking in Indonesian toddlers

G Course of the Eye Affection

Xerophthalmia is not a condition of which the course can be easily determined The victim usually is an ambulatory patient possibly a babe in arms Every slight affection of the cornea is to be considered as an emergency As the condition is not painful the mother may not worry much about it nor does the child complain Once he is seen at the out patients department he has to be cured at once thus barring observation of the spontaneous course

A certain degree of photophobia apparently dependent on the inflammatory reaction present impels the child to keep its eyes shut and so withdraws them from observation On Java Bitot spots are sometimes considered to be a sign of worms a neither alarming nor infrequent condition Literate adolescents may complain of amblyopia and ask for glasses

Xerosis of the conjunctiva with or without Bitot spots may be present for months without much inconvenience to the victim An incidental school inspection or the sequels of an accident at dusk may cause their discovery Bicknell and Prescott (1953) suggest that prolonged mild deficiencies lead to formation of the spots Acute and chronic are rather deceptive conceptions as the lesion may be progressive or regressive Simple xerosis is a rather chronic condition if untreated In acute cases such as occur after measles no Bitot spots may develop In possibly mild very chronic deficiencies opacity and hyperpigmentation without spots dominate the picture In another chronic case the type of spots illustrated by Roels *et al*

(1958) which they call intermediary develops It is a localized affection of the conjunctiva hyperpigmented thickened but not rough which is crowned by a small plaque of scum If there is enough abnormal secretion if it is sticky enough if the underlying surface is rough enough if the subject does not blink too frequently if there i no inflammation the foamy deposits develop

Usually both eyes are affected but rarely to the same degree This applies to all stages of the affection Sweet and K ang (1935) report that in 8 % of cases one eye only was involved and in 24 % the affection of the second eye sometimes came weeks after the first It may be that the phenomenology largely depends on delicate variations in the condition of the tissues

Whether the xerosis once present progresses or disappears depends on many factors The more acute cases develop in the wake of an acute infectious disease and may vanish a few weeks later even if small perforations of the cornea have occurred and without further treatment They give the impression of a transient deficiency The nutritionally purer cases are rather obstinate Then xerophthalmia should be considered as a chronic relapsing affection Although the physician cannot radically change the dietary habits of the subject he may at least achieve that in the course of the vulnerable years no permanent los of vision occurs What may happen even to a more or less supervised child may be illustrated by an example

Case history 10/27/52 male age 30 months Intermittent visitor of polyclinic now for fever The last but one of five living children Delivered by trained midwife Father is tobacco peddler earns about three shillings a day Weight at 2 months 4500 gm at 6 6500 gm at 18 7500 gm at 24 6800 gm now 7000 gm Six visits before this four times for broncho pneumonia twice for diarrhea At 20 months xerosis conjunctivae with Bitot spots cured with vitamin A injections At 22 months relapse of xerosis same treatment At present again xerosis conjunctivae and corneae Bitot spots no ulcers Present state hemoglobin 8 8 gm % depigmentation muscle atrophy enlarged liver slight edema Liver biopsy heavy steatosis Mantoux at 18 months and now negative at 1/100 No malaria Fair load of *Ascaris* Diet breastfed till 12 months Had cow s milk irregularly Mother insists that the child daily had green vegetables

In the remainder of the cornea or in big leucomas excerbations of the original condition may be observed (Fig 10) In a series of 64 consecutive patients mentioned in Table IV eight presented maculas or leucomas as evidence of earlier damage by the affection besides the fresh lesions observed at first examination The e considerations are of course relevant

10 C

11

only if the eye lesions are not too severe In that case the usual end is death even if by timely treatment the local process has been arrested

There seems to be no doubt that prexerosis xerosis conjunctivae xerosis corneae and keratomalacia are stages of increasing severity of the same condition But some manifestations may be skipped or be obscured by an attending inflammation The older the patient the more chronic the course is not a rule without exceptions An infant less than a year old may have only conjunctival xerosis with Bitot spots but more often it will be observed at an advanced stage of keratomalacia The suggestion of Fillat (1939) that in a child corneal xerosis lasts only hours does not apply to the majority of the Indonesian cases which are toddlers Keratomalacia may occur at every age but in adults it is rare and is conditioned by a



PLATE IV

Sequel of xerosis corneae perforans

Fig 10 Male 3 years old left eye Large perforations with r proap e in a corneal scar of a rel p ı g case (eral hat e of cor a still ı t et

Fig 11 *Same case* right eye Old leukoma adherens caused by a xerophthalmia episode one year earlier

Fig 12 Female 3 year old S bt tal leukoma originated by measles after 6 weeks treatment Note bulging pore of Meibom gland (P tie t ıs] sh n Fig 78)

definite disease whereas in the infant it depends rather on elementary deprivation

H Anatomy

The pathogenesis of xerophthalmia has been approached from very diverse angles The condition has been ascribed to a primary desiccation by keratinization of the epithelium (Wolbach and Howe 1925 1933) or to a secondary desiccation resulting from decreased function of the lachrymal gland (Mori 1922) or to diminished *mucus production by the* goblet cells (Collins 1930) A different viewpoint was that of Yudkin and Lambert (1923) who considered xerosis to be a sequel of local inflammations of the conjunctiva Less connected with histological changes was the idea of Browning (1931) that a toxin caused the affection of the cornea of Findlay (1925) that lack of lysozyme an anti infectious component of tears preceded it or of Mellanby (1934) that xerosis was secondary to loss of neurotrophic control by the trigeminal nerve Parts of the syndrome could be thus explained but most of these theories failed to furnish adequate arguments for the causation of the whole

However the characteristic metaplasia of the conjunctiva and cornea offers a ready explanation of the symptoms observed The conjunctiva has a polymorphous mostly squamous epithelium consisting of many layers in which many polygonal or cuboidal and goblet cells occur In xerosis the disappearance of the mucoid goblet cells is a constant symptom (Kreiker 1930) Herbert (1898) and Courbieres (1935) believe that this is the primary lesion which causes both the keratinization and the xerosis The inferior layers show extensive ballooning of cells with crowding of the abundant pigment to the top (Damean Gillet 1938) There is a tendency to discontinuity of the tissue by the formation of big intercellular lymph spaces The surface consists of loose keratinized desquamating cells that have lost their nuclei The deeper epithelial layers abound in keratohyaline granula According to Kreiker it is not so much the dyskeratosis which causes the irregularities of the surface as hyaline degeneration of the sub epithelial layers By bursting through the superficial keratinized tissue thi causes a villous (*zottig*) appearance Kreiker insists that if the surface does not burst no Bitot spot ensues This gives a ready explanation of those cases showing typical xerosis *without development of spots* (Fig 8) Some authors stress the occurrence of fatty infiltration both within the epithelial cells and in the deeper layers (Marinosci 1930 Hamada 1930 1933) *Disappearance of mucus presence of abnormal* fat and cellular debris probably produce the peculiar secretion and furnish the substrate for development of xerosis bacilli

The pathological process in the cornea is said to progress from infiltration through necrosis to ulceration. It may well be, however, that necrosis is the primary lesion, followed by infiltration and, in the case of mummification, desiccation. The destroyed corneal mass looks at times like a clear jelly, then again turbid and yellowish. It has been compared with a coagulation necrosis (Wright 1922). There are not many good reports on the anatomy of keratomalacia *sensu stricto* in man. Even at the rare autopsies only the final stage of histological alteration could be studied.

The local ulceration present under epithelial defects may quite well correspond to the pictures described in rats. There is a demarcation zone of polynuclear leucocytes through the whole of the stroma. Moore (1957) suggests that it even develops without evidence of infection. It is probably a reaction to the epithelial change (Mori 1922). This would correspond with the thick gray halo of infiltration that surrounds ulcers of patients treated with vitamin A (Fig. 9). In the diffuse process Jaensch (1927) observed necrosis of the epithelium, infiltration by leucocytes, and a typical fatty degeneration. The last factor may give a clue as to the difference between local defects and keratomalacia *sensu stricto*. It could indicate a cornea more diffusely deteriorated than the one affected by local ulcerations, where the usual defensive mechanisms still prevail. In the cornea affected by mummification Pillat (1939) states that the eight to ten superficial layers of epithelial cells are still demonstrable. Any sign of inflammation or softening is lacking. In rats Wolbach and Howe (1925) observed keratinizing foci in the original differentiated epithelium and replacement by less specific epithelial cells from the basal layer. A more detailed discussion of the differences between the localized perforations and the diffuse decay of the cornea would seem very desirable in view of the great differences in prognosis as to vision.

I. DIAGNOSIS OF XEROPHTHALMIA

Xerosis of the conjunctiva preceded or accompanied by hemeralopia and reacting favorably within a week on administration of a reasonable quantity of vitamin A can hardly be mistaken for anything else. Still it is often hard to decide whether an increased degree of dryness and wrinkling is a specific symptom or an effect by external or hereditary agents. Dryness and hyperpigmentation then are more useful guides than increased vascularity or thickening indicated by a more pronounced relief of the interpalpebral conjunctiva. A subjective interpretation may fake a high incidence, but the absence of serious stages in the environment should then cause doubt. A localized swelling like a pingueculum or a pterygium ought never produce difficulties in differential diagnosis.

Klaften (1923) stated the hemeralopia in pregnancy never was accompanied by xerosis Birnbacher (1927) observed a xerosis in 77% of European children under 10 years suffering from hemeralopia between the ages of 10 and 20 years the percentage for males was 30 and for females 18 Hemeralopic women more than 20 years old never suffered from xerosis Absence of hemeralopia in a toddler with xerosis would make the specific nature suspect Though it is sometimes difficult to prove the observer very often receives sufficient evidence without determination of dark adaptation Dhanda (1956) quotes a number of cases of many years duration without nightblindness It is difficult to imagine that this refers to authentic cases of xerophthalmia

The distribution of the xerotic parts may be diffuse or patchy Diffuse or cloudy pigmentation especially in the juxtalimbar areas often appear to be pathognomonic Not every pigmentation in colored people is related to the condition Patwardhan (1952) reported on a circumcorneal ring or slender sickle *somewhat separated from the limbus as an independent* sign There may be other types according to race The reactive pigment is especially to be sought for in the eyeslit Pillat (1929 1931) believes that it serves to protect the nuclei because of its intracellular localization It is also present within the cornea and Pillat even suggests that necrosis in *keratomalacia is connected with the structural inability of the cornea to* produce sufficient pigment The differential diagnosis depends on its transient nature But it takes weeks and possibly months before it is driven away by therapy Thomson (1953) supposes that it may persist as a permanent brown staining That exposure to light is a contributing agent may be proved by the striking pigmentation of the bulbar conjunctiva in squinting children in whom a larger part is exposed

The *Bitot spot* is a very characteristic phenomenon in xerosis It may however be absent and its shape and consistency depend very much on the quality of the substrate and the nature of the pathological secretion by which it is produced As an inert substance it does not itself react on cure with vitamin A This takes more time than the restoration of the epithelium manifested by the disappearance of dryness and opacity For diagnosis neither a typical xerosis nor a Bitot spot cause much difficulty Apparently Bitot like spots occur in conditions of the eye without interference by vitamin A Sie Boen Lian (1938) reported on such spots (see Section IV A) in those of Agricola (1905) they were caused by a localized congenital keratinization Roels *et al* (1958) used the Bitot spot as an indicator of hypovitaminosis A in a survey in Ruanda Urundi Their results demonstrate the suitability of the spots as a criterion for assessing the vitamin A status of groups in combination with serum levels Klerks (19 6) con

cluded that the Bitot spot is a valuable sign of malnutrition because of the significance of the relation with typical signs of malnutrition

Although Nicol (1949) in connection with the scarcity of vitamin A in the diets of Nigerian farmers refers to several eye changes it is not clear what proportion of these pertains to the classic symptoms of xerophthalmia If contrasting evidence may be u ed the thickening of the bulbar conjunctiva and pigmentation of the sclera (of the conjunctiva bulbi?) did not belong to the specific affection since they were present in consumers of diets either poor or rich in carotene but nightblindness and corneal opacities were numerous among the groups with the lowest consumption of carotene Thickened conjunctivae occur in adults of many colored races especially in individuals much exposed to the sun Without the typical unwettability and opacity the diagnosis of xerophthalmia is not dependable A distinction between the condition in children and adults could probably have proved the point

Prexerosis is not so easy to establish especially in children In kwashiorkor ca es the conjunctiva is usually thin and translucent but there is often a decrease in humidity and a trace of wrinkling Oomen and Malcolm (19 8) observed in malnourished Papuan children a imilar proneness to dryness which was not accompanied by hemeralopia and was absent in better fed counterparts Slitlamp examination could in such cases have furnished the clue Thus in Mexico Pagola (1948) found diminished humidity of the cornea in 90 % of the eyes of kwashiorkor patients he examined xerophthalmia with ulceration in 7 % and Bitot spots in 1 % In children suffering from trachoma a certain degree of dryness is usually present but then the specific symptoms betray the real nature McLaren (1956) states that pingueculae have frequently been confu ed with Bitot spots although this is difficult to understand to one who has een both lesions as they differ in many ways

Xerosis of the cornea without epithelial defects is a short lived symptom except when it changes into mummification Diagnostic pitfalls in semicomatose or moribund patients in which the factor lagophthalmos is evident can easily be avoided Early infiltrates may look nummular Ulcers may be confusing Ulcus serpens with hypopyon is not rare in measles In case of doubt the presence of xerosis conjunctivae may decide in favor of xerophthalmia The rapid cure by vitamin A adds further proof In view of the limited facilities in the Tropics it will usually be more sensible to use the argument *ex juvantibus* than to search for keratinization in scrapings of the conjunctiva

In contradistinction to former opinions (Pillat 1939 Bietti 1940 May *et al* 1940) it should be emphasized that neither the *serum lev l of vitamin*

A nor that of carotene is very helpful in establishing a diagnosis Though in case of xerophthalmia a decrease of both is typical even if the range is rather wide approximately the same low values are found in kwashiorkor (Trowell et al 1954 Behar et al 1958) The levels especially in apparently normal children in a vulnerable environment are not much higher The most that can be said of serum levels of vitamin A in xerophthalmia is that they are never high and rarely average that they are lowest in cases of keratomalacia that in groups of xerophthalmia cases the averages are lower than in healthy controls (Table VI) Their significance is about the same as that of protein levels in subclinical protein malnutrition cases In short the biochemist especially in an endemic environment cannot help us decide whether an individual child has xerophthalmia

Malnutrition keratoconjunctivitis Partly because of difficulties in establishing the diagnosis of keratomalacia but mostly for general clinical reasons the eye affection described by Blumenthal (1950) in malnourished Bantu children and young adults deserves close attention When the author calls it a sociological disease and considers it as the commonest cause of preventable blindness in South Africa a clinician familiar with such terms in South East Asia will prick up his ears But if the principal alterations are described as excessive wetness of the cornea and vascularity of the conjunctiva accompanied by lachrymation and mucus liquefaction he will realize that this must be a radically different affection even if corneal perforations are common The description of the clean prolapse and the clinical uniqueness that the cornea should dissolve away quietly and insidiously at one small point without a sign in the eye to attract superficial attention of the parents suggests that there is no connection with xerophthalmia It is stated that Bitot spots never were observed The type of button mushroom iris prolapse and the more rarely occurring nipple type staphyloma are incompatible with the disseminated irregular perforations and their sequels in xerophthalmia The localization is initially stromal or mesodermal but not epithelial

The young victims are said to be fat and podgy to win prizes at baby shows A reference to the motto of this paper may suffice to stress the difference with keratomalacia patients

Blumenthal apparently has also been troubled by the term keratomalacia Possibly the name keratomalacia was originally given to a single eye condition but has been used indiscriminately to describe a hotch potch of conditions in the Middle and Far East many of them no doubt dietetic in origin In our opinion the indiscriminate use of the term keratomalacia has mostly been in the sense of an overstatement but it was not difficult to recognize definite trends and similarities in the descriptions reviewed here

from East or West On the other hand it is felt that the designation of the African condition by Blumenthal is an understatement and does not quit come up to the essentials of the description To avoid confusion with an other malnutritional eye affection already beset by terminological trouble we propose to call it *keratolysis profunda*

TABLE II
COMPARISON OF XEROPHTHALMIA WITH KERATOCONJUNCTIVITIS BLUMENTHAL

	Xerophthalmia	Keratoconjunctivitis
Surfaces	Dry p gmentation	Wet vascul rization
Initial lesion	Epithel al	Stromal
Pitot spots	Common	Absent
Iris prolapse	Through damaged surface	Through clean surface
Fi al l ion	Nebula leucoma stapl yloma phtisis	Leucoma nipp e staphyloma
State of nutriti n	Mal utri ion evident	Fat podgy baby
Preferred age	Toddler	Chil l until puberty

TABLE III
DISTRIBUTION OF AGE AND OCCURRENCE OF XEROPHTHALMIA ON JAVA COMPARED WITH KERATOCONJUNCTIVITIS IN THE EAST CAPE PROVINCE

Age (years)	Xerophthalmia %	Keratoconjunct t [b] %
1–5	83	33
5 15	9	34
15 30	5	16
O er 30	3	17

Total number of cases con dered 5991
[b] Total number of ca e considered 175

In Table II the principal differences between Blumenthal s disease and xerophthalmia are summed up and in Table III a comparison is made of the age distribution for both conditions in the respective endemic regions

V The Patient

the cli ical picture of keratomalac a is not yet exhau te l by the symptom whi l ha g en the di ease t nam

MEYER AND NA SAU (1930)

Bloch (1919) and Blegvad (1924) the first investigators to study the condition on a large series of patients after the striking di covery of the fat soluble factor called it *dystrophia* alipogenetica and *dystrophia* xeroph thalmica re pectively Mori s hikan was a Japanese children s disease with diarrhea during the summer months Van Stockum (1938) contended

that therapy aiming exclusively at treatment of the eye was doomed to failure De Haas *et al* (1940) stated that dystrophy and xerophthalmia go hand in hand while the vicious circle is closed by infections Finkelstein (1948) distinguishes between pure cases of keratomalacia in which the isolated deficiency of vitamin A is clear and between cases with grave general disturbance which anamnestically are not connected with lack of vitamin A The latter he states are much more frequent

Is xerophthalmia then merely the ocular manifestation of a systemic disease of grand style of a generalized affection of the ectoderm as emphatically propounded by Pillat (1939)? A superficial examination of an average patient may impress the observer by the marked wasting of muscle (Figs 13 and 14) a more intensive examination by the striking degree of fatty infiltration of the liver (Figs 17 and 18) Neither tissue has to do with the ectoderm Muscle wasting or fatty infiltration of the liver do not even belong among the diverse lesions in many tissues and organs which according to Moore (1957) are due to vitamin A deficiency Or is it that affections of other tissues throughout the body of which the specific avitaminotic nature is well known collaborate in creating the miserable condition of the patient with advanced xerophthalmia? Of these lesions those of the skin deserve attention because the integument is the most closely related ontologically to the affected epithelia of the eye

A The Skin in Xerophthalmia

A few questions must be answered here regarding the skin abnormalities present in some patients with xerophthalmia First Are they a typical manifestation of hypovitaminosis A that is are they only to be found in connection with this deficiency? Second Are they a constant symptom?

A condition called *phrynoderma* by Nicholls (1933) has often been stated *to accompany xerophthalmia and to react favorably to repletion* with vitamin A It was originally defined as a papular dry skin eruption frequently accompanied by a mild neuritis and (or) eye symptoms such as nightblindness dimness of sight xerophthalmia or keratomalacia the patients are very liable to diarrhoea or dysentery when this occurs the neuritis becomes more marked a high mortality results The disease is due to vitamin A deficiency but other food factors may be at fault

It was observed originally in (adult) prisoners in a Ceylon jail The eye lesions called keratomalacia were present in 8 % of the cases and started by injection of the vessels running from the inner and outer canthi of the eye to the cornea the redness of the vessels stands out in marked contrast to the whiteness of the sclerotics at the same time yellowish

thickenings occur where the vessels disappear at the corneal sclerotic junction

The next change is the appearance of pinpoint opacities in the cornea these increase in size and may become two or three millimetres in diameter Then the opaque areas ulcerate and if the ulcers do not heal they become extensive and hypopyon develops and finally the anterior chamber and cornea become disorganized and permanent blindness follows

Before the opacities of the cornea have appeared many of the prisoners complain of dimness of vi ion and even of blindness The visual symptoms must be due to the deficiency affecting other parts of the eye than the cornea

These symptoms are not in accordance with the typical qualities of xerosis or with the reactionless sloughing of the cornea in keratomalacia Nicholls perhaps prematurely compared the syndrome with *mandama* a disease occurring in young children almost synonymous with mira mus and concludes Although neuritis is not mentioned it is probably the same condition as occurs in prison There is no doubt however that xerophthalmia representing a typical manifestation of hypovitaminosis A was responsible for the blindne s in 66 % of the pupils of a school for the blind and deaf in Ceylon and in many of the low age instances mentioned by Nicholls (1934) Whether their phrynoderma can be ascribed to exactly the same cause remains dubious

Somewhat later Loewenthal (1935a) reported on 44 cases of xeroph thalmia and 277 of phrynoderma in 1000 school pupils in Uganda He used the term xerophthalmia in the same sense as it is employed in this paper Reviewing Nicholls interpretation of the evidence of hundreds of cases occurring in Negro school children Loewenthal (1935b) rejected neuritis and diarrhea as symptoms of vitamin A deficiency but positively included phrynoderma and xerophthalmia

Since then the neuritis component (including burning feet and pro gressing into ataxia with loss of reflexes) has aroused further attention by the frequent wide scale occurrence of the syndromes of deafness ataxia and retrobulbar neuropathy in European pri oners of Japanese internment camps in the Far East They also presented shark skin and similar af fections protein malnutrition was very marked but xerophthalmia was absent

Gopalan (1947) showed that phrynoderma could be cured by admin istration of linseed oil in conjunction with the vitamin B complex Menon *et al* (1950) proved that administration of a fat rich in essential fatty acids improved phrynoderma and increased the iodine value of the serum lipids An ordinary hospital or boarding school diet may do the same Even if

treatment with vitamin A sometimes gives results this may not be proof enough as the reaction is much slower as compared with the restoration of the eye lesions To say the least the connection of the dyskeratosis of the skin with vitamin A is less apparent than that of the exposed tissues of the eye in xerophthalmia

Nicol (1949) distinguished between folliculosis as a simple enlargement or undue prominence of hair follicles (keratosis pilaris) which may occur on either a healthy looking or a xerotic skin He uses follicular hyper keratosis to describe a marked thickening of hair follicles with projecting spiny plugs of keratinized material occurring superimposed on a general ized xerosis (the phrynoderma of Nicholls) He does not find a correla tion with carotene intake in folliculosis It is however marked with xerosis follicular hyperkeratosis and elephant skin The protein intake of his subjects was satisfactory but there was a high incidence of liver dis turbance in all groups the groups with low carotene also consumed little fat

Without discussing further the specificity of phrynoderma (see also Stannus 1944) we refer to papers denying the coincidence of phrynoderma and xerophthalmia especially in toddlers (Aykroyd and Rajagopal 1936 Frazier *et al* 1943 Baptist and de Mel 1955 Klerks 1956 Oomen 1958)

Besides the coarse follicular phrynoderma *finer more diffuse types of folliculosis and dry atrophic desquamative alterations* have often been described (Nicholls 1933 Pillat 1939) They are common in toddlers with either kwashiorkor or xerophthalmia even in the immediate neigh borhood of the eye (Fig 6)

Radhakrishna Rao (1937) studied the histopathology of the skin in adult cases of keratomalacia not showing phrynoderma He suggests that the latter is a result of a pathological response to external irritation and is not of an essentially different nature

A reference to *exfoliative disorders* of the skin in severe malnutrition of toddlers (crazy pavement skin) and vitamin A deficiency has never been made They appear also among the cases described by Mori (1904) and were illustrated in one of Bloch s papers (1931) Oomen (1953 1954) could not discover any difference in prevalence whether such a child had xerophthalmia or not

The frequent association of atrophic and dyskeratotic skin alterations connected with protein malnutrition in young and adult subjects has not always received sufficient attention from authors concerned with specific skin change In our opinion protein malnutrition often dominates the pos sibilities created by isolated deficiencies

Conclusion It may be concluded that these disorders of the keratinizing parts of the epidermis do not essentially belong to the syndrome of xerophthalmia The apparent variables are vitamin A protein and fat in the diet and age of the subject but it is risky to indicate a closer association Therefore it may not be justifiable either to include the bulging pores of the Meibom glands in xerophthalmia among the more specific signs of hypovitaminosis A It may in fact be advisable to dissociate the types of dyskeratosis seen simultaneously with minor variations in xerophthalmia in protein malnutrition in pellagra (besides the typical skin manifestations) (Gillman and Gillman 1951) and in scurvy (Fox 1941) from the realm of specific deficiency They probably can be judged more profitably from the Gillmans viewpoint of nonspecific regulation patterns

It is unfortunate that the skin constituting as it does a considerable portion of the body mass and representing an accessible ectodermal epithelium as well does not furnish useful information on specific changes caused by lack of vitamin A Our disappointment becomes still greater when we try to unravel clinically the connection with other tissues and other deficiencies

B Coincident Alterations of Other Tissues

Except the skin most of the biologically essential surfaces of the body are difficult to study Mostly from evidence from animal experiments we know that the epithelium of the urinary tract of the intestine and of the respiratory organs often is affected by a similar metaplasia

The desquamative derangement of the first stressed in English cases by Spence (1931) of which we would expect to find evidence in the urinary sediment often cannot be proved (Sweet and K ang 1935 Oomen 1954) A bladder calculus is not a common finding during the active eye lesions and in juvenile cases of bladder calculus the proof of a vitamin A deficiency is usually lacking (van Stockum 1938) Despite attention devoted to the subject in Indonesia only once in a 6-months old child has the coincidence with renal calculus been described (Sampoerno 1926)

Bicknell and Prescott (1953) suggest a connection between renal calculi and hypovitaminosis A due to deficiency previously caused by a kidney affection This is not a problem for which clinicians in regions where xerophthalmia is endemic would seek a conclusive interpretation Their problem particularly pertains to the high incidence of bladder calculus in small boys (Oomen 1958)

Diarrhea not always ascribable to a definite microbial agent has been mentioned by many authors Ramalingaswami (1948) described a number of xerophthalmia cases of which the diarrhea did not respond to sulfaguanidine but reacted promptly to administration of a concentrate of

vitamin A Sixteen out of twenty nine cases from a series mentioned by Oomen (1954) suffered from a severe diarrhea (eight with intermittent steatorrhea) which in some cases still persisted after the dysenteric role had been ruled out

Bronchopneumonia is a common complication in most cases The variety most closely connected with xerophthalmia is apparently an interstitial pneumonia The expected bronchiectasia is difficult to prove by simple means As indirect evidence Pillat (1959) reports the immediate reaction of bronchitic symptoms to administration of vitamin A We have to assume on the strength of a few autopsies (Blackfan and Wolbach 1933 Sweet and Kang 1935) that keratinization and metaplasia of the respiratory and bronchial epithelium must be considered as potentially grave lesions

It is not too difficult to explain away the clinical symptoms connected with the condition of these different types of epithelia by ascribing them to lack of vitamin A However as in the case of the skin lesions it is embarrassing to prove the point

Returning to our original question we must conclude on clinical evidence that respiratory and intestinal disturbances are more close and ominous partners of xerophthalmia than are changes of the integument or the urinary tract According to Moore s (1957) experience whole groups of rats exposed to vitamin A deficiency may die of pyelitis and cystitis This at least has no apparent equivalent in man The bad condition of the lungs or of the intestine could explain at least to some extent the deterioration of the nutritional state On the other hand neither if caused by common infection induces such a sad state so rapidly

A rat showing the typical signs of vitamin A deficiency would exhibit xerophthalmia eventually associated with emaciation A pig would have normal eyes and weight but not be able to stand on its hind legs (Moore 1957) In man the most common combination would be affection of the eye together with a variable state especially in toddlers of nutritional dystrophy

The abnormalities elsewhere in the body of a victim of xerophthalmia produce as much evidence as they add confusion in interpreting the general affection of health

C Relationship with Protein Malnutrition

Many accounts from all parts of the world early as well as recent indicate that the apparent coincidence of xerophthalmia and malnutrition has not received adequate attention Several lesions of the eye have been associated with starvation malnutrition or protein depletion (Bietti 1950) Excepting *xerophthalmia* the most important perhaps are a *polymorphic*

superficial keratopathy described in Greek adults under war conditions (Djacos 1942 Petzetakis 1950 Spyratos and Petzetakis 1943) eye affections of internees during the last war in the Far East in which neural lesions dominated exemplified by *retrobulbar neuropathy* (Nicholls 1951 Oomen 1959) and the conspicuous syndrome delineated by Blumenthal (1950) in South Africa as *malnutrition keratoconjunctivitis*

Of the four the connection between malnutrition (stressing the general and infantile aspects) and xerophthalmia seems to be closest It is even demonstrable between the degrees of affection of conjunctiva and cornea and of the deterioration of the nutritional state Using an index involving the state of the skin and of the muscles the size of the liver the presence of edema and the degree of growth retardation (Oomen 1955) single conjunctival xerosis can be proved to be most frequently associated with a slight degree of malnutrition whereas a severe disturbance occurred simultaneously with the perforating complications of corneal xerosis and with keratomalacia (Table IV)

TABLE IV

Degree of Nutritional Disturbance Related to Stages of Xerophthalmia in 64 Children Less Than 6 Years Old (Java)

Degree of xerophthalmia	Malnutrition		
	Slight	Moderate	Severe
Xerosis conjunctivae	14	34	14
Bitot spot	12	2	5
Xerosis corneae simplex	4	15	2
Xerosis corneae perforans	1	8	12
Keratomalacia	0	0	8
Total number of children	14	34	16

Positive Mantoux test positive malaria smear and enlarged spleens excluded

Regarding this category of Indonesian urban children one might wonder which *type of malnutrition* is most prevalent among those affected by xerophthalmia We consider here as kwashiorkor the variety with preponderate edema and still retaining some caloric reserves and as marasmus the condition typified by shriveling of the skin muscles and fat tissue by anhydremia etc Of the 64 infants and children less than 6 years old presented in the table 8 manifested the typical syndrome of kwashiorkor (Figs 15 and 16) 7 were typically marasmic (Figs 13 and 14) and in 5 of 7 infants less than 1 year old anhydremia was a striking feature (Fig 19) the remaining 42 were in a state comparable to marasmic kwashiorkor or syndrome policarencial infantil (Autret and Behar 1954)

In short The child with xerophthalmia suffers from the same types and degrees of malnutrition as his counterpart free from visible manifestation



PLATE X

Nutritional state and xerophthalmia

Fig. 13. Male, 2 years old. Marasmus. Xerosis corneae perforans.
Fig. 14. Male, 1 year 3 months old. Marasmus. Xerosis conjunctivae et corneae.

of hypovitaminosis A. Oomen (1953, 1954) compared the state of the liver in biopsies of 79 cases of infantile malnutrition in two Indonesian towns. 54 exhibited xerophthalmia and 25 were free from such symptoms. The incidence and the degrees of steatosis and fibrosis were essentially similar in both groups. Hoogenkamp (1956), studying rural toddlers in Borneo, had the same experience. There is very little reason—if we disregard the eye signs—to consider xerophthalmia as a special variety of malnutrition. There are strong reasons to regard every degree of xerophthalmia, except possibly incipient alterations, as a case of general malnutrition or nutritional dystrophy.

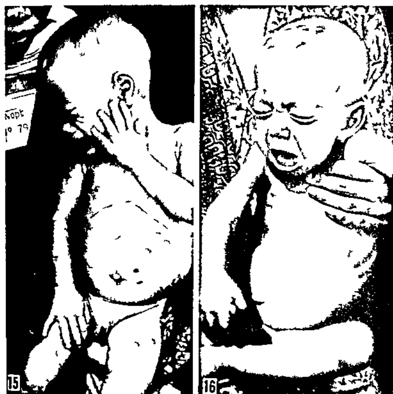


PLATE V (*Continued*)

Fig. 15 Male 1 year old Kwashiorkor Xerosis corneae perforans

Fig 16 Male 1 year 6 months old Kwashiorkor Xerosis corneae perforans hypopyon

The picture suggested here concerns the small child affected by xerophthalmia as found in Indonesia Recently it has been analyzed further by Poey Seng Hin (1957) The earlier reports of Mori (1904) at the turn of the century in Japan come close to this picture Several of the descriptions from India and Malaya (Achar 19 0 Sen 19 4 Gopalan 19 6 Said 1955) also resemble it At least some of the cases of Bloch and Blegvad belong in the same category There can be little doubt that grave disturbances of the diet or the mother's diet are at the root of the trouble It is however somewhat different from the case of Pillat patients who were of the disprivileged class of Chinese apprentices and companions in



PLATE VI

Nutritional state and xerophthalmia

FIG 17 Female 3 years old Liver enlargement Xerosis conjunctivae et corneae

FIG 18 Male 1 year 1 month old Liver enlargement Xerosis corneae perforans

distress Their age their nutritional history and their mode of reaction to stress by hypovitaminosis A may explain at least part of the differences

The association of xerophthalmia with protein malnutrition is so frequent and so close that we cannot help wondering which is the primary lesion Is it admissible since this denomination of a disorderly syndrome is so much in vogue to speak of xerophthalmia combined with kwashiorkor as an *associated vitamin deficiency?*

Protein metabolism is apparently and very plausibly involved in every nutritional disturbance at the level of the active protoplasm or of the living cell mass The significance of the turnover rate often has been stressed in



PLATE VI (*Continued*)

Fig 19 Female 11 months old Anhydraemia cachexia Keratomalacia

Fig 20 Female 2 years old Weak thin child con[illegible]

Note tears

this respect At the time that kwashiorkor and allied syndromes had not yet obtained clinical recognition the term most often used in connection with xerophthalmia was dystrophy Either from a nosological or from a public health viewpoint *xerophthalmic dystrophy* is in all cases excepting the initial affection a better designation of the syndrome than is *xerophthalmia* alone The disorder though characterized by the lesions of the eye in fact is a syndrome and not an incidental symptom The pathology progresses along pathways different from those in pure kwashiorkor though the final stages resemble each other very much (Table X)

It is not very attractive to start a new racket in relation to the vitaminic interpretation of xerophthalmia Vitamin A has successively been

acclaimed as a promoter of growth a shield against infection a guardian angel of the epithelium or a partner in business of the ectoderm None of these claims have been materialized satisfactorily in later years Still arguing clinically one could defend with some success the statement that vitamin A protects against protein malnutrition

TABLE V
TENTATIVE CHARACTERIZATION OF KWASHIORKOR AND XEROPHTHALMIA IN SMALL CHILDREN

	Kwashiorkor	Xerophthalmia
Serum level		
Vitamin A	Low	Low
Carotene	Low	Low
Albumin	Low	Low
Globulin albumin ratio	Low	Low
General symptoms		
Dyskeratosis skin	Yes	Yes
Muscle wasting	Yes	Yes
Steatosis liver	Yes	Yes
Growth retardation	Yes	Yes
Dy pigmentation	Diffuse depigmentation	Bulbar hyperpigmentation
Peak incidence	18–24 month	36–48 months
Sex distribution	Equal	1 4 Male to 1 female
Eye symptoms		
Conjunctiva	Thin dry white	Unwettable opaque thick Bitot spots
Cornea	No alteration	Unwettable opaque defects
Glands (lachrymal sebum)	Parenchyma affected	Ducts affected
Mucous cells	Less affected?	Specific affection
Associated disease		
Predisposing	Less specific	Measles hepatitis acute malaria tuberculosis
Intercurrent	Dysentery broncho pneumonia	Same

Nobody disputes that the vitamin is essential for maintenance of health or for the stabilization of tissues but its essential role eludes the experimental biologist (Handler 1958) There is little indication that the mechanism of action in scotopic vision is connected with this vitamin s more general function of maintenance of the epithelial tissues (*Nutrition Reviews* 1959) A suggestion of Dowling and Wald (1958) on the relationship seems useful in this context The protein opsin and the outer segments

of the rods in the retina are stabilized by combination with retinene and deteriorate rapidly in its absence The authors suggest that many tissues contain structural proteins which are stabilized by combination with vitamin A and that these also deteriorate in a deficiency state

The conception that the action of vitamin A ultimately is to stabilize structural proteins indeed may find confirmation in the clinical aspects of xerophthalmia in man The general manifestations of protein malnutrition—retardation of growth disturbed keratinization and atrophy of the skin wasting of muscle respiratory and intestinal symptoms fatty infiltration of the liver and edema—produce a clinical syndrome which in no way differs from the former aspect Advanced xerophthalmia is always accompanied by nutritional dystrophy but there is a category of severely malnourished children which does not show any sign of xerophthalmia They are present in an environment which produces many cases of xerophthalmia and they represent the majority in surroundings where vitamin A is not involved

Within the limits of their significance the serum levels in both confirm the close connection In clinical malnutrition many levels connected with diet are low including albumin and vitamin A levels in apparently normal counterparts similar figures may be found There is no regular correlation between serum levels and the clinical state in protein malnutrition though in a rough way for instance in averages of groups they correspond (Table VI) Low levels are a rule but the connection is not close A child with 20 IU of vitamin A may or may not have xerosis During treatment the serum levels may not yet show a rise while the effect of vitamin A administration is already demonstrable in the eye lesion A child with kwashiorkor may have no signs of xerophthalmia but still present low levels of vitamin A (Scrimshaw *et al* 1956) On administration of the vitamin the serum levels may not rise on feeding extra protein and omitting the vitamin the serum levels of the vitamin may rise (Arroyave *et al* 1957)

In one particular case only a mixture of amino acids was given for 18 days to a 14 month old case with kwashiorkor Besides the serum protein levels vitamin A and carotene increased (from 6.4 to 21 μg and 1.0 to 3.0 μg respectively) but the values for vitamin B_1 B C and E decreased (Behar *et al* 1958)

The effects of vitamin A depletion are violent in infants with keratomalacia The disturbance of the general state then is acute just as in case of infantile beriberi The frequent occurrence of keratomalacia in endemic surroundings suggests that often the child has low reserves and the breast milk low contents at least the latter has been confirmed As in kwashiorkor the toddler is the most characteristic reactor but the slower exhaustion of

reserves—apparently slower than those of the protein pool—causes the clinical manifestations to occur at a later period In the adult the relation is more difficult to establish because of the frequent presence of liver lesions in his case the dystrophy is sometimes not so pronounced

TABLE VI
AVERAGE SERUM VITAMIN CAROTENE AND PROTEIN LEVELS AND RANGE IN NORMAL AND IN AFFECTED CHILDREN

Classification and number	Average age (years)	Carotene (IU %)	Vitamin A (IU %)	Albumin (gm %)	Globulin (gm %)
Normal (16)	8 5 (7–10)	120 (66–205)	67 (18–104)	3 8 (2 8–4 7)	3 3 (1 8–4 0)
Xerosis conjunctivae (10)	4 5 (1½–10)	29 (0–63)	18 (0–49)	3 4 (2 5–4 3)	3 4 (2 3–4 4)
Xerosis corneae I (10)	4 (1 8)	7 (0–20)	3 (0–10)	2 7 (1 9–3 4)	3 5 (2 5–4 7)
Xerosis corneae II (10)	2 5 (1 5–4)	—	9 (0–23)	3 0 (2 5–3 7)	3 2 (2 8–4 0)
Keratomalacia (10)	1 7 (1–3)	—	7 (0–21)	1 7 (1 5–2 1)	2 6 (1 9–4 0)

[a] Normal children are apparently healthy subjects from a poor area at Jogjakarta which regularly produced cases of xerophthalmia In the group with xerosis conjunctivae the cornea was not affected The groups xerosis corneae I and II designate various stages of corneal affections including perforations See also Tap Hie Tiong (1956)

With exception of some cases of keratomalacia in young infants and of some affections in adults the clinical sequence is first eye signs afterward general malnutrition signs Clinically it is beyond doubt that the eye signs are primary as regards hypovitaminosis A Theoretically connected with the less applicable evidence from animal experiments structural changes of a general kind could precede them and create metabolic havoc which is not yet clinically demonstrable If this supposition is correct xerophthalmia should be considered as one of the precursors of protein malnutrition comparable to the protein catabolism in acute infectious disease Hence it is evident that in a combination of xerophthalmia and kwashiorkor there would be no associated vitamin deficiency in the latter but associated protein deficiency in the former To prove the hypothesis that protein malnutrition is a sequel of vitamin A deficiency it would seem necessary to demonstrate catabolic processes before they can be caused by protein malnutrition

The arguments as to why xerophthalmia could frequently occur in protein malnutrition have been summed up by McLaren (1958) as possibly con

nected with (1) deficient absorption of vitamin A or carotene by lack of enzymes (2) deficient conversion of carotene (3) deficiency of blood constituents able to carry the combination free alcohol-albumin in the blood (4) impairment of liver storage and (5) disturbed utilization of the vitamin at the cellular level. However another possible mechanism recently came to the fore (6) The *differences in vitamin A destruction power of red blood cells* in cases of pure kwashiorkor and of kwashiorkor with xerophthalmia respectively (Gopalan 1959).

Though every one of the former five aspects would merit careful consideration even the confirmation of all would fail to explain why so many cases of kwashiorkor and allied syndromes do not show a trace of recognizable hypovitaminosis A. There is no lack of arguments epidemiologically why xerophthalmia can occur. The diets involved are usually poor in fat protein vitamin A. Carotene conversion manifestly constitutes a special drawback. But children in some parts of the world develop only one type of kwashiorkor while in other regions two categories appear the first being free of eye symptoms the second presenting impressive eye damage. Up till now the only further distinction between the two groups is that in the latter the specific sex distribution of xerophthalmia is demonstrable and that it occurs in a later stage of the development of the child.

D. Correlation with Infections

It is often extremely embarrassing to decide in a patient whether infections of the bronchial system or of the intestine are a cause or symptom or a sequel of hypovitaminosis A manifested by the eye affection. All three possibilities may occur in an environment where vitamin A is a limiting factor. There are however a number of acute contagious diseases which regularly appear as a determinant. The symptoms then impress as a more or less direct sequel of the infection. A disease which often has been associated in this regard is measles: it was first mentioned by Fischer (1843). Of a series of 46 cases Hiro and Yamada (1936) mention 9 with measles; the eye lesion appeared four times in the first week, twice in the second and thrice in the third to fourth week. The previous diet had not always been strikingly deficient in vitamin A. The xerosis of conjunctiva and cornea appears when the remnants of the exanthem still are present (Fig. 23). The incidence depends of course on the periodic appearance of the virus. A wave of measles in Indonesia sometimes is followed by a minor one of xerophthalmia (van Munen 1938). The explanation is probably that an already precarious balance is suddenly deteriorated by a disturbance of the storage (Woo and Chu 1940). Incidental observations of an accompanying rapid increase in liver size proved by biopsy to be caused by fatty infiltration



PLATE VII

Infectious disease and xerophthalmia

Fig 21 Female 4 years old Tuberculosis xerosis conjunctivae et corneae
Fig 22 Male 3 years old Chickenpox xerosis conjunctivae et corneae

may visibly suggest a decreased storage space Of the contagious virus diseases of childhood chickenpox (Fig 22) and hepatitis can be added out of personal experience In the case of whooping cough the picture is already more complicated by the marked influence of decreased food intake An infection in which epithelial alteration are only distantly included but liver affection is probably important is acute malaria (Fig 26) Primary phase tuberculosis is an example from another category It differs from the later stages in that it often behaves as an acute disease

Of these examples it could be stated with some reservation that they do not produce so often and so rapidly a state of general malnutrition as do amebic and bacillary dysentery in which the chronic or recurrent tendencies especially in malnourished persons are well known Chronic tuberculosis and relapsing malaria—in China kala azar—are undermining dis



PLATE VII (*Continued*)

FIG 23 Female 3 years old Measles xerosis conjunctivae et corneae hypopyon
FIG 24 Female 9 years old Chronic malaria annular liver cirrhosis xerosis conjunctivae (Eye is shown in Fig 4)

eases of a different category tending to produce anemia malnutrition and xerophthalmia either together or separately

The specific influence of some of these diseases would find an explanation in observations on serum levels of vitamin A Acute febrile infection cause a sharp decrease normal levels are restored after the temperature becomes normal again Chronic infections also cause a decrease but the restoration is slower and more irregular (McCreary and Tisdall 1953) Even in adult healthy subjects artificial hyperpyrexia for 2 hours only causes a highly significant decrease of serum vitamin A level (Mendez *et al* 1959)

Sweet and K'ang distinguished between (1) *primary or predisposing disorders* represented by chronic illnesses (2) *associated or correlated disorders* in their case (Peiping) rickets in 40% of the children (3) *sec*

ondary *complicating diseases* said usually to be acute infections They mention upper respiratory tract infections otitis sinusitis *bronchitis and* bronchopneumonia but suggest that some of these belong to the first group Actually 2 of 6 of their autopsies were on children 6 months to 6 years old who had suffered from measles Because of the supposedly present alterations of the tissues an acute infection like a bronchopneumonia with pyrexia *may indeed be called secondary* Of an acute *contagious virus infection* shared with *many others it is more likely that the illness* acts as a trigger than as a predisposing disorder Another argument for the provoking action of some acute infections is that the eye symptoms appear rapidly and sometimes disappear without specific therapy It may be relevant that these virus infections act at the cellular level on the other hand the possibility *that the primary effect may be pyrexia* alone should not be ruled out It may be well worthwhile *to establish by comparative* studies whether some infectious diseases have a specific action on vitamin A availability in the tissues or whether the loss or unavailability of the vitamin ultimately is a sequel of protein deprivation In Table V the clinical similarities relations and dissimilarities of xerophthalmia and kwashiorkor have been tentatively reviewed

Pillat (1939) insisted that *fever in adults with keratomalacia* was a characteristic *phenomenon* On administration of cod liver oil or vitamin A preparations it disappeared in 8–10 days Sweet and K ang (1935) probably in *the same category* of patients reported that it was not usually present In children suffering from serious xerophthalmia pyrexia would find a plausible interpretation in the infections present *if it is lacking* as *in some severe or terminal* stages this would indicate a grave metabolic disturbance In fact during convalescence bouts of fever hitherto absent may indicate a return to normal reactions Several times we observed for instance a relapse of vivax malaria or the change of a Mantoux reaction from negative to positive although a fresh infection could be *excluded*

Reviewing the evidence produced either by clinical *observation* in man or by the extensive *experience* in animals we have *to conclude that* hypovitaminosis A is not *primarily* a systemic disease of the ectoderm or the epithelium but a metabolic disorder The predilection for these specific tissues and for the eye tissues especially may be *explained by* the involvement of the vitamin in cellular structure or in stabilization *of structural* proteins

Infection either locally as a reaction to epithelial lesions and possibly extending later on to become a general influence or extraneous systemic infection both acute and chronic may well be explained by the decrease of reserves or the disturbance of metabolism *It is evident that not only* the physiology of vitamin A is *involved but* also that of protein

The difference in phenomenology between protein and axerophtol malnutrition may ultimately depend on the turnover rate of protein in the former and the maintenance of structure in the latter

1 Blindness and Xerophthalmia

Though many children die an equally considerable percentage goes blind or semiblind The latter condition is another expression that the lesions are often asymmetrical Leucomas staphylomas and phthisis bulbi are the tale telling sequels in later life Of 152 infants with xerophthalmia at Djakarta reported by de Haas *et al* (1940) 44 became wholly blind and 25 blind in one eye In a series of 298 survivors of Blegvad's cases 27 % went blind 24 % had a greatly reduced vision in both eyes and 35 % in one eye and in only 14 % recovery of both eyes without any reduction in vision resulted Much depends of course on the stage at which the patient is seen by the physician Postponement of treatment for 1 day may make all the difference It is not so much skillful treatment that the patient needs as administration of vitamin A in time by the first person who suspects the etiology Still many mothers do not realize what is happening behind the closed eyelids of the toddler who hardly complains and if trained help is far away or expensive it is the child who is going to pay for the omission with his vision Blindness and mortality are steady partners in the aftermath of xerophthalmia hence it is difficult to judge numerically the catastrophic consequences An idea of the bearing on the problem of blindness in n[illegible] ern Sumatra appears from the figures of van Manen (1938) who extended expert ophthalmological care far into native rural areas Among the more serious eye affliction he mentions the following number of cases atrophia bulbi 204 staphyloma anterior 65 macula corneae 527 keratomalacia 115 trachoma 407 cataract 235 pterygium 74 chorioretinitis 60 Of the first four categories probably most of the cases would be or have been connected with xerophthalmia Of 605 semi blind he ascribed 132 to this affliction of 489 totally blind 123 Very much similar figures on the causation of blindness in the state of Kelantan (Malaya) have been reported by McKitterick on (1956)

1 Mortality and Xerophthalmia

The condition may be present for months without much disturbance in a normal schoolchild but in infants with keratomalacia mortality is alarmingly high As there is a more or less direct relation between the degree of the eye lesion and the state of the child (more so than in adults) mortality will vary with the severity of the affection Of the examples mentioned 35 % of de Haas (1940) cases died and of Blegvad's (1924) 24 % As

without adequate help a child in the stage of perforations of the cornea often would not survive for more than a few weeks we realize how big the share of annual mortality can be attributable to this cause if in a given population 1 % is found to be affected by the more serious lesions

As to the lapse of time between the appearance of the corneal lesions and death which in his infant cases was 4–15 days Blegvad (1924) remarked that the shortest survival time was in the oldest infants The vulnerability of the cornea declines with increasing age so that it may easily be supposed that the necrosis of the cornea with the somewhat older children does not appear until the last stage of the xerophthalmia—shortly before death This supposition does not apply to adult cases who often survive (Pillat 1939) Neither does it concern localized perforations in corneal xerosis of toddlers It may indicate a special pattern which applies to keratomalacia in infants

VI The Diet

Much as we know about the dietary factors by which artificially specific lesions can be caused in experimental animals usually our knowledge about the diet which precedes the manifestation of xerophthalmia in man is limited and vague This is very regrettable because the pattern of development of man being unique his digestive physiology cannot easily be matched by animal counterparts and the sociology of the acquisition and preparation of his food is very difficult to imitate in the laboratory

Although in environments where only sporadic cases occur the emphasis is on metabolic disturbance it is diet which is most important in regions or period with endemic xerophthalmia The difference cannot be exactly delineated In the former isolated cases occur induced by an abnormal diet in the latter secondary metabolic disturbance is by no means to be neglected There is little cause to insist on the composition of diets which cause eye symptoms in patients with liver cirrhosis cystic pancreas fibrosis or coeliakia More important from a health viewpoint is the alimentary background of tropical living conditions Why do some environments produce many cases and are they rare elsewhere?

Mori (1904) explained the hundreds of cases that he observed in Japan by the sudden transition from breast milk to a diet deficient in fat which especially prevailed among rural children producing most of the cases The efficiency of cod liver oil in curing the condition suggested the idea of a fat deficiency Diarrhea he stated would aggravate the lack by decreasing resorption Mori noted that the diet usually consisted of barley not even of the better rice which contains relatively much fat flour and vegetables but no cow s milk or meat In the coastal regions where fishing is prac

ticed the affection was of course a rarity This type of diet should be remembered because we will meet it again Very likely it was rich in carbohydrates poor in protein and fat with no real vitamin A sources present but still containing carotene

A PRIMARY DIETARY DEFICIENCIES

In the extensive series of observations on xerophthalmia of Bloch (1919 1931) and Blegvad (1924) we are confronted with the first accurate study of the diet of the subjects Bloch believed that a common factor in all cases was that the patients for a more or less extended period had been deprived of whole milk His most significant observation concerned the development of 8 cases within one month in ward B_1 of a children s home

Ward B_1 and B_2 each accommodated 16 infants The diet consisted of breakfast—oats gruel with biscuits or beer soup with some whole milk lunch—porridge or buttermilk soup oats gruel fruit soup and now and then meat soup with barley besides mashed potato with fish or minced meat tea—cocoa with bread and margarine supper—porridge and bread with margarine The milk used for the preparation of the porridge and cocoa was partly defatted (*halbentfettet*)

The only difference between ward B_1 and B was that the nurse in charge of B_1 gave oats gruel and biscuits for breakfast and the one of B beer soup and whole milk The nurses were allowed *in case of diarrhea* to omit the beer soup (plus whole milk!) In ward B five children did not increase in weight during winter in ward B_1 ten In May eight of the ten in ward B_1 aged 1½ to 4 years were afflicted with xerosis Two had recently suffered from measles one from bronchopneumonia one had whooping cough and two had tuberculosis So it was not alone a deficient diet which preceded the eye affection!

The remaining details make it clear that the children probably had enough calories and protein but that their food was poor in fat vitamin A and carotene The diet resembled an experiment of several months duration From other cases too it is apparent that the only probable source of vitamin A for many Danish children at the time of World War I was whole milk not vegetables and that this had been substituted for skimmed milk As a confirmation of the experiments of Osborne and Mendel (1921) it was a most illuminating experience

It does however differ radically from the dietary conditions prevailing in the Tropics responsible for the frequency of xerophthalmia In European surroundings it was practically always the bottle fed infants or the toddlers on an institutional diet who were affected In Japan China India and Indonesia few infants are or were bottle fed and cow s milk with or with

out fat is not part of their dietary inventory Albeit in a meager quantity these infants receive preformed vitamin A only with breast milk

B CORRELATION WITH CAROTENE INTAKE

Apparent correlation The correlation of the incidence of Bitot spots seems acceptable in some studies of Aykroyd and Rajagopal (1936) in South India and of Klerk (1956) in Indonesia Both compared the intake of school boys with the presence of signs of malnutrition in poor and better off surroundings The former authors found 6 3 % in a poor school and 1 % in a better class school Klerks found 4 5 % and 0 7 % respectively In both studies the staple food was milled rice with small quantities of vegetable and little or no milk meat and eggs The most striking differences were with respect to calories protein—especially animal protein—fat calcium and vitamins A B and C It should be noted that in Klerks cases these intakes were matched *in the averages of groups* with corresponding values for vitamin A and albumin in the sera

Van Manen (1938) compared two regions in northern Sumatra In one a community of recent immigrants subsisting mainly on laboriously transported rice and dried fish xerophthalmia was rife among children On the island of Nias however among a poor population consuming yellow sweet potatoes as a staple food instead of rice no evidence of the disorder was discovered

Doubtful correlation In a group of 436 children aged 1 to 12 in a number of labor camps Aykroyd and Krishnan (1936) observed symptoms of xerophthalmia in 27 % The diets consumed by year groups 1–5 5–8 and 8–12 were estimated to contain about 450 µg 710 µg and 780 µg of carotene respectively They concluded It follows that optimum requirements of carotene must exceed that of the diets prescribed and the establishment of a sub minimal figure of carotene intake associated with deficiency symptom may be of use in practice

On the other hand van Veen *et al* (1937) found that similar intakes of carotene as only source of vitamin A in the diets of adult prisoners were sufficient to produce serum levels of carotene and vitamin A comparable to those of well fed Europeans

In another investigation in pupils of residential hostels Aykroyd and Krishnan (1937) observed 0–23 % of subjects with Bitot spots Supposing that the spots were a manifestation of avitaminosis they were not able to discover a relation between vitamin A activity of the diet and the incidence of the eye lesions Possibly the children already showed the same lesion at admission resulting from deficient feeding in the earlier years of childhood The institution where the highest incidence was found

(B_4) was a home for destitute orphans In the hostel in which the vitamin A activity of the diet was lowest (vitamin A 14 µg carotene 200 µg) no cases of Bitot spots were observed In others cases were present despite an intake of 1500 µg even with the inclusion of sheep s liver once or twice a week The diets of both examples are stated to include about 80 gm of protein and 25 gm of fat daily (see Table XII)

Conditioned correlation Certain results obtained during extensive surveys in rural West Java may illustrate the difficulties in collecting adequate information on the link between xerophthalmia and diet (Sagalaherang report 1940) One of these concerned a district where many cases of night blindness and xerosis had been reported to occur A fortnight later the population and especially the children were examined At that date eleven (7 %) of the cases originally reported had died and of the children examined twenty four (60 %) died shortly afterward It is very probable that most of the affected had suffered from dysentery and half of them still were sick (fever dysentery edema bronchitis) It is worth noting that in the families examined 75 of the deceased children had died between 1 and 5 years of age and that 58 of the child mortality in the district was ascribed to intestinal affections

Afterward the diet was studied in families of children presenting xerosis and Bitot spots and in families without these affections The gross result are mentioned in Table XII (subhead IV b and c) In two villages the per capita diet of ten affected families contained each 280 µg of carotene (range 80–500 and 170–380 µg respectively) and of ten families without evidence of Bitot spot 400 and 250 µg (range 100–660 and 150–330 µg respectively)

The diets of the two groups did not differ significantly The diet of individual children could not be determined but in samples the carotene sources (mostly leafy vegetables and sweet potatoes) were consumed less by small children In cases of diarrhea the diet was often limited to rice only Many mothers were plantation workers in their absence an older sister would supervise the nutrition of the small child

It is tempting to complete this story Under normal conditions the low intakes suggested by the figure would yet be sufficient to prevent xerosis and hemeralopia The low fat intake and the absence of preformed vitamin are unfavorable factors In periods of stress such as that caused by a wave of infectious diarrhea the low reserves of vitamin A are exhausted and the consumption absorption or conversion of carotene is disturbed This may be the case even when far greater amounts of carotene are consumed than in the instance cited

In a study of the diet of particular groups that regularly produced a

TABLE VII

BITOT SPOTS IN RELATION TO AVERAGE DIET IN SCHOOL AGE CHILDREN[a]

Locality	Number	Bitot spots	Vitamin A (μg)[b]	Carotene (μg)[b]	Protein (gm)	Fat (gm)	Calories
I South India							
Hostel B 4	61	++	50	1400	80	30	2800
Hostel B 5	31	+	230	1800	70	55	2800
Hostel B 9	185	+	0	500	50	15	2000
II Indonesia							
Urban high class	115	—	460	2600	50	30	1500
Urban middle	86	+	70	1100	40	15	1100
Rural poor	254	—	0	3700	10	7	1000
III Indonesia							
Physician's children	25	—	290	1330	70	35	2200
Orphanage	33	+	0	2600	35	11	1500
Poor area	21	+	0	530	30	8	1300
IV Indonesia							
Good region	(a)	—	0	850	50	8	2100
Poor region	(b)	+	0	280	30	4	1250
Poor region	(c)	—	0	350	35	5	1450

[a] Abbreviated data. Assessment of diet: I, food as purchased; II, consumption estimated by interview; III, actual consumption; IV, family average consumption.

[b] 1 IU vitamin A = 0.3 μg; 1 IU carotene = 0.6 μg.

I. Residential hostels for boys in Madras Presidency (*Aykroyd and Krishnan, 1937*).

II. Schoolboys in western and central Java (Klerks, 1956).

III. Children in central Java (*Tjap Kie Tiong and Soekartijah, personal communication*).

IV. Regional dietary surveys: (a) 0.5% Bitot spots in children of 108 families; (b) ten families with 9% Bitot spots in children; (c) ten families without Bitot spots (Patyt and S gal lerang Reports, 1940).

number of xerophthalmia cases throughout the years at Jogjakarta (Tap Kie Tiong 1956) the potential intake of vitamin A was sometimes as high as 2070 µg or as low as 530 µg per day All the vitamin A activity had to come from carotene The average intake of a control group of physicians children among whom no evidence of xerophthalmia could be discovered was 1330 µg but 23 % of it was preformed vitamin A (Table VII subhead III)

C Correlation with Liver Function

In an accurate survey Nicol (1949) tried to relate the intake of Nigerian farmers to evidence of vitamin A deficiency One group consumed eight times as much carotene (3100 µg) mostly from sweet potatoes and mangoes as the two others and twice as much fat (44 gm) There was a striking absence of corneal opacities and hemeralopia in the former The incidence of liver cirrhosis in all three groups was high (15 12 and 7 %) and the correlation was so high particularly for corneal opacities as to make it doubtful whether vitamin A deficiency could be considered as the primary cause of the lesions A possible explanation is that liver disturbance is an inhibitor of carotene utilization and that corneal opacities would develop at a low age if this was suboptimal

D Correlation with Fat and Protein Intake

A superficial review of many poor diets of the Tropics shows that they are usually low in protein and fat Hence it is natural to suspect that these aspects may be coresponsible for the availability of vitamin A from carotene When considering the relation of protein to carotene utilization we do not get very far before becoming involved in the apparent link between vitamin A deficiency and protein malnutrition (see Section V C) On reviewing the evidence of the dependence on fat moieties of the diet a valuable hint may be derived from the relative absence of xerophthalmia in many African regions where red palm oil is an important dietary item Carotene is there offered in greater quantities and in a more utilizable form (Wilson et al 1937) than in the diets containing cooked vegetables and little fat common in many other regions

It is interesting to note that Andre and Ganzin (1954) believe that an abundance of fat and of carotene in the diet could protect even against protein malnutrition In relation to diets containing 27 gm protein 86 gm fat and 37 000 µg (!) of carotene daily they found absence of the kwashiorkor syndrome high blood levels of vitamin A (254 IU) and carotene (874 IU) and signs of *hypervitaminosis A* in children

Surveys of per capita consumption offer little explanation especially t e

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IV Indonesia							
Cool region	(a)	−	0	850	50	8	2100
Poor region	(b)	+	0	290	30	4	1250
Poor region	(c)	−	0	350	35	5	1450

Abbreviated data Assessment of diet I food as purchased II consumption estimated by interview III actual consumption IV family average consumption

[b] 1 IU vitamin A = 0.3 μg 1 IU carotene = 0.6 μg

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IV Regional dietary surveys (a) 0.5% Bitot spots in children of 108 families (b) ten families with 9% Bitot spots in children [illegible] (c) [illegible] families without Bitot spots (Patjet and S galal erang Reports 1940)

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Surveys of per capita consumption offer little explanation especially for

a problem of toddler xerophthalmia as it exists in Indonesia Oomen *et al* (1954) tried to assess the diet of a toddler population in a poor district of Djakarta by determining consumption frequencies Subsistence conditions there were limited and monotonous Three per cent of the children suffered from xerophthalmia including serious degrees The diet of malnourished subjects proved to be much inferior to that of their healthy partners The consumption of protein sources (fish soybean and peanut products) and carotene rich foods (vegetables yellow fruits) was much better in the fourth year of life than in the second These precious food stuffs even if present in the adult diet were not yet given to the youngest The per capita consumption of carotene was estimated at 330 μg per day Except in 10% of the children who received irregularly small quantities of egg and milk no vitamin A was consumed

TABLE VIII
PERCENTAGE OF SPECIAL FOODSTUFFS BESIDES RICE IN THE DIET OF 82 URBAN CASES OF INFANTILE MALNUTRITION

Diet	Xerophthalmia (51)	No xerophthalmia (31)
Daily		
Bread	40	50
Sweet potato[b]	35	35
Cassava	35	35
Soy products	40	45
Banana	55	65
Breast milk[c]	20	25
Regularly not daily		
Meat	30	30
Fish	20	15
Green gram	20	40
Amaranth[b]	40	20
Other green vegetables[b]	35	40
Yellow fruits[b]	25	30
Cow s milk	10	6

In Djakarta (Oomen 1953)
[b] Carotene sources
Vitamin A sources

In another survey a trial was made to distinguish between the diet of malnourished toddlers with and without xerophthalmia (Oomen 1953) The result was essentially negative (Table VIII) As to the questionable provision with vitamin A it is interesting to note that one third of the subjects with xerophthalmia consumed carotene sources regularly

E. Comments on the Value of Carotene

The first to remark that carotene in appreciable quantities occurred in the diet of xerophthalmia victims in Indonesia was Tijssen (1936). His observations regarded rural children (Atchin). He was so impressed with the experience that he spoke of "apraxia" of the (pro) vitamin and made a distinction between keratomalacia in breastfed infants as a deficiency disease and xerophthalmia in toddlers as a constitutional disease, because the latter occurred only in some of the carotene consumers.

People not consuming milk, milk products, eggs, or liver have to produce their vitamin A from carotene. If they do not consume green vegetables or yellow fruits, etc., the way is open to manifestations of deficiency. For some small dependent children or for people not having access to vegetables, this is a plausible explanation.

As carotene in the green Tropics is omnipresent and is consumed in varying quantities, the problem for the majority of the cases may not be the absolute quantities but the efficient utilization of this vitamin A source. If this is less than expected from theoretical considerations, it may seem practical to raise the recommended allowance up to an ill-defined safety margin, but the scientific approach would be to find out the reason for the poor utilization. This is a problem of eminent importance in poor environments and one to which hitherto little attention has been paid. The preceding studies in special groups or in closed communities give some idea where to look for solutions, but a valid answer can most probably be provided only by individual diet studies. On diets containing reasonable quantities of carotene, fat, and protein, the occurrence of xerophthalmia is very unusual. It is apparent that in many individuals a meager quantity of carotene suffices to protect against xerophthalmia, whereas in some a relatively large quantity fails to do so.

Though there are obscure or controversial points regarding the less prosperous tropical areas, the dietary aspects of xerophthalmia there are not a problem of preformed vitamin A intake, but they are partly one of availability of carotene and partly a problem of utilization of this provitamin.

F. Prosperity and Reserves

An indirect indication that among people who are able to buy the more expensive foods like tinned milk, eggs, and meat, xerophthalmia does not occur may be derived from a comparison of the incidence in patients of the private practice and of the polyclinic of the eye hospital in Jogjakarta. Whereas the polyclinic produced 400–500 cases yearly during the last five years, not a single case was present among the case histories from private

practice Mori (1904 1922) had a parallel experience in Japan In the year that the consultation fee was raised the percentage of cases treated was considerably below the average Courbieres (1935) stated that hemeralopia (in France) s arrete devant le galon A similar remark was made by Louwerier (1923) about nightblindness in plantation laborers in Sumatra. In young newly imported Javanese nightblindness was rather common it vanished as they became better off It is interesting to note that in countries where xerophthalmia is endemic this situation exists This precludes to some degree any considerable influence of genetic factors on the prevalence of the condition

Erratic and indirect the dietary evidence may usually be yet there is one conclusion that we may safely draw In areas where vitamin A is regularly consumed no endemic xerophthalmia exists

The civilian European internees during the Japanese occupation of West Java had to subsist after a period of two years of slowly increasing scarcity *for eighteen months on a diet of less than 1400 calories—less than 15 gm* protein and less than 5 gm fat on an average A certain amount of cheap greens was included but animal products were rarely consumed Thousand of cases of edema and pellagra developed few under 10 years of age No case of xerophthalmia was reported not even in the children born at the *beginning of the internment period Their early reserves were* apparently sufficient to cover a stretch of several years

VII Occurrence

A Age Distribution

Consideration of one of the outstanding aspects of the epidemiology of xerophthalmia the relation to age is evidently essential for understanding the nature of the affection

The patients of Bloch (1919 1931) and Blegvad (1924) in Denmark were characteristically infants less than 1 year old Those of Mori (1904) and Oomen respectively in Japan sixty years ago and in Indonesia now were *toddlers Those of Pillat (1929 1930 1931 1939)* and Sweet and Kang (1935) in China were young adults

In an environment where hypovitaminosis A is endemic infants toddlers school children and adults suffer *but the affection rate differs widely* Pregnant women usually considered vulnerable apparently suffer *least*

The epidemiology of xerophthalmia involves the consideration of the life span We should remember that large reserves more than are of apparent use are stored in the normal liver of adults It is hardly possible to exhaust them by omitting vitamin A or carotene from the *diet in human*

experiments to such a degree that xerophthalmia ensues. The shortest time required to achieve incomplete depletion as indicated by serum level in adults in the Sheffield experiment (Moore 1957) was 400–500 days. In view of this we must conclude that the vitamin A status of many persons in the poor regions in the Tropics, at least in certain age groups, must differ fundamentally from that of regular consumers of the preformed vitamin in prosperous regions. This may be one of the fundamental aspects of the difference in nutritional state between well fed and some ill fed populations.

It seems natural to divide the types of xerophthalmia according to certain aspects of etiology. In practice this boils down to age groups.

B. Infant Xerophthalmia

Many authors insist that this type is severest and most acute. An example of the distribution as to degree at low age from Jogjakarta is given in Table IX. Most of the observed cases are keratomalacia, but with diligent inspection less serious cases can be observed. The etiology in the first year of life is rather varied.

TABLE IX

Severity of 38 Consecutive Cases of Xerophthalmia According to Age (Jogjakarta)

Degree of xerophthalmia	Age (in years)			
	1	5	6–14	1
Prexerosis	0	0	1	2
Xerosis conjunctivae + Bitot spots	2	3	3	
Xerosis corneae	1	5	0	0
Xerosis corneae perforans	1	9	0	1
Keratomalacia	3	2	0	0
Total cases	5	24	4	5

Least difficult is the interpretation in artificially fed infants (Fig. 2). The cases of Ploch (1919, 1931) are classic examples. They represent the rarer instances of a primary deficiency. Such cases may occur in the Tropics if carbohydrate foodstuffs are given instead of breast or cow's milk. In some towns of Indonesia artificial feeding was responsible for the bulk of cases of xerophthalmia observed in infants (van Stockum 1938). A special variety was represented by the scores of cases in Indonesia before the last war when sweetened condensed skimmed milk appeared on the market which because of the low price ousted the tinned whole milk (de Haas *et al.* 1940).

There are however breast fed children who within a short time develop keratomalacia. Sometimes their mothers show Bitot spots but cases have



PLATE VIII

Infants with xerophthalmia and after healing

FIG 25 Male 5 months old Left eye panophthalmia right eye keratomalacia and phthisis bulbi Partly breastfed child

FIG 26 Male 25 days old Keratomalacia after primary malaria Breast fed child

been described also in infants of mothers in perfect health (Goll 1942 Corcos *et al* 1954) In a few keratomalacia was already present at birth (Kumbaur 1922 Maxwell 1932) As both are exceptions we will forego their interpretation Our impression is that in environments where xerophthalmia is rife the number of breastfed infants affected is relatively large Prematures and twins are regularly present among the cases In a few the mother suffered from liver cirrhosis or the condition developed after malaria or tuberculosis (Fig 26)

The mothers in question must derive their vitamin A also from carotene In two of three breastfed cases of keratomalacia of de Haas and Meulemans (1938) the mothers milk did not contain any vitamin A in the third there was 14 IU the blood of the mother containing 18 IU The average content of mature milk of Indonesian and Chinese mothers was 60 IU per 100 ml comparable mean values for European mothers in Indonesia would be 225



PLATE VIII (Continued)

Fig 27 Male 2 month old Xero... corneae ... (illegible)

Fig 28 Female 3 years old Recovered after measles ... in both eyes lost (Eyes shown in Fig 12)

IU (Meulemans and de Haas 1936) in England 144 IU (Kon and Mawson 1950) in the United States 258 IU (Lesher *et al* 1945)

The not infrequent affection of the breastfed infant in endemic areas may therefore be ascribed to low birth reserves or to low vitamin levels of the breast milk More difficult to explain is why so many infants on low intakes do not develop eye lesion

C Toddler Xerophthalmia

In many countries breastfeeding is continued well into the second year Even under adverse circumstances the suckling infant of a healthy mother is then usually protected against xerophthalmia Toward the end of the first year cases not having a special etiology are often connected with insufficient breast milk and unbalanced carbohydrate addition For practical reasons we will consider here as toddler xerophthalmia the cases occurring in the second and including those in the fifth year Epidemiologically this

is the most important and homogenous variety There is a peak in the third and fourth year This is later by nearly two years than the peak incidence of kwashiorkor (Oomen 1955) The type of xerophthalmia considered here is an affection of toddlers who have been breastfed for a considerable time Most probably this is the epidemiological variety which is of utmost importance in South and East Asia It probably comprises 80-90% of all cases and although not always as severe as infant xerophthalmia it causes more harm more deaths more blindness and more leucomas A person going blind or semiblind at the age of 3 or 4 years in certain environments almost certainly becomes so because of xerophthalmia They are the subjects of the grisly pictures of Tijssen (1940) from rural Atchin and the keen observer can meet them daily in every town market or among the dock workers of Indonesia

It is difficult to indicate why there are controversies among authors about the highest prevalence being among young adults (Sweet and Kang 1935 Pillat 1939) It may depend on their point of vantage whether at inspecting schools or in charge of a children s ward or of an eye department A slight affection often would not impel the patient or his caretaker to seek medical help and therefore would go undetected On the other hand it is laborious indeed to screen a population seeking for a few per cent affected though this already indicates a serious health problem

The special burden of the toddler in respect to xerophthalmia as distinct of that of the infant was demonstrated by Mori (1904) in Japan A comparison (city data) between the endemics in Denmark and Japan respectively clearly indicates this particular aspect As regards Indonesia three independent series are accounted for each essentially showing the same tendency (Table X) Smaller series from India (Kirwan and Sen 1941 Sen 1954 McLaren 1956) produce similar evidence

Common or probably common to all these groups is a diet without milk and eggs with a limited quantity of mostly vegetable protein and fat and a varying intake of carotene If red palm oil is popular as in many regions of Africa the problem is apparently absent On the other hand in Ruanda Urundi where it is not popular Roels *et al* (1958) studied a xerophthalmia problem which was not infrequent in older children and adults but severe degrees were not observed in toddlers by the ophthalmologist of the team

A study of the relation of the diets in various age groups to serum levels of protein vitamins A and E and carotene could perhaps help explain the preference for the toddler period As a rule vitamin A and carotene levels are low in the young and increase slowly with age An example in different prosperity groups of Central Java is given in Table XI

Rice occupies an outstanding position in the diet of the countries of southern and eastern Asia It is often present as a very exclusive staple food and if the obvious alternative to vitamin A is not taken the potency

TABLE X

PERCENTAGE DISTRIBUTION OF XEROPHTHALMIA ACCORDING TO AGE GROUPS

Origin[a]	Number	Infants to 1 year	Toddler 1–5	school age 5–15	Young adult 15–25	Old adult 25+
China (1)	203	15.0	17.0	0	41.0	20.0
Indonesia (2)	6300	4.4	73.0	13.6	3.8	3.9
India (3)	36	3.0	83.0	8.0	3.0	3.0
Indonesia (4)	890[b]	5.4	88.2	6.4		
Indonesia (5)	166[b]	2.0	80.0	18.0		
Denmark (6)	476[b]	87.0	11.0	1.0		
Japan (7)	1475[b]	2.2	74.8	23.0		
Japan (8)	46[b]	0	76.0	24.0		

Number in parentheses refer to the following sources

(1) Sweet and Kang 1935 (2) Oomen 1957 (3) McLaren 1956 (4) Oey Khoen Liang 1955 (5) van Wisselingh, personal communication 1958 (6) Blegvad 1924 (7) Mori 1904 (8) Hiro and Yamada 1936

[b] Groups of children only

TABLE XI

INCREMENTS IN SERUM VITAMIN A WITH INCREASING AGE WITHIN GROUP OF DIFFERENT PROSPERITY (CENTRAL JAVA)

Age	Number	0–50 IU %	50–100 IU %	100–150 IU %	150+ IU %
Physicians families[b]					
5–15	22	0	3	3[illegible]	36
15–25	18	0	6	44	0
Farmers families					
5–15	29	69	31	0	0
15+	50	18	52	30	0

Sex distribution about equal

[b] Satisfactory diet see Table VII subhead III

Less satisfactory diet fair carotene no axerophtol (Gunung Kidul van Veen and Lanzing 1940)

of the diet must be very low It may not give the mothers or their newborn infants adequate liver reserves or it may in some way hamper the utilization of carotene

A curve similar to that of the incidence of xerophthalmia could often be produced by plotting infectious disease in toddlers relative to age We remind the reader that xerophthalmia is a steady partner of the acute and

early chronic infections of childhood On the other hand this is a universal influence whereas the prevalence of xerophthalmia apparently varies

The distinct giant wave of cases in the toddler period suggests that there must be a definite widespread agent in some regions and not in others The rather late appearance of the bulk of xerophthalmia cases suggests that the reserves at birth and the acquisition by suckling last a long time and probably are not compensated before the child obtains a more independent diet The shape of the frequency curve does not conform to the rate of growth and therefore does not indicate that at this age there should be a requirement *higher than that dictated by body mass*

D SCHOOL AGE XEROSIS

The more manageable school child provides an easy subject for health examinations including those for xerosis But if the age distribution is *similar in his environment to the typical examples shown above the* observer will miss the most serious manifestations of the affection The characteristic lesion of the school child is xerosis conjunctivae with *Bitot spots* At that stage his health will not usually be poor Obviously children with perforated ulcers cannot attend school

Still the school child with Bitot spots is some kind of indicator of the vitamin A status of the community He is able to and often will complain *about nightblindness He is more mobile in contrast* with the affected toddler who hides at home

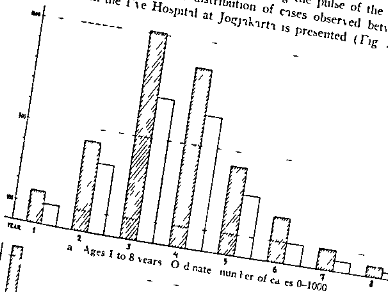
E ADULT XEROPHTHALMIA

Although serious degrees including keratomalacia occur at any age *they are definitely rare after puberty* So far as the clinical history of the subject is known the prevalence of liver disease among adult cases is striking This often involves patients who have or have had icterus On the other hand nutritional edema or emaciation in adults is a less constant companion to the affection As a whole the nosological background in regions of endemicity does not differ except in quantity very much from that in cases reported in well off regions

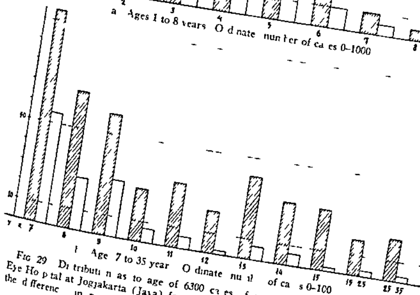
An apparent exception is a large portion of the accurately studied cases of Pillat Sweet and Kang (1935) and others in China Sweet and Kang state that *the increased incidence in the second and third decades can be* explained by the number of soldiers and apprentices seen with the disease They constituted approximately 40 per cent of our patients and together with students comprised 90 per cent of those in the second and third decades of life In the studies of Loewenthal (1935a) no precise dis

tinction as to age was made in the hundreds of cases observed (50 % of school children and 87 % of adults examined were affected)

It is clear that an exact knowledge of the quantitative aspects of xerophthalmia in relation to age is useful in feeling the pulse of the vitamin A status As an example the distribution of cases observed between 1934 and 1954 in the Eye Hospital at Jogjakarta is presented (Fig 29 a and



a Ages 1 to 8 years Ordinate number of cases 0–1000



b Age 7 to 35 year Ordinate number of cases 0–100

Fig 29 Distribution as to age of 6300 cases of xerophthalmia observed in the Eye Hospital at Jogjakarta (Java) from 1935 to 1954 Hatched bars = males Note the difference in preponderance of the male sex in low age and older groups

b) The condition is well known in the region The origin of the patients reflects the distribution of inhabitants of the urban districts as well as of the densely populated rural regions Most patients come on their own incentive Less serious degrees in older patients would perhaps not so easily find their way to the hospital The ophthalmologists in charge have for generations been interested in the condition As far as hospital statistics can be impartial this one for our purposes seems free of disturbing bias (Oomen 1957)

Of 6300 case histories studied 267 were those of infants less than 1 year old 4715 toddlers 1–5 years old 866 school age children 5–15 years old of which 483 were 5 or 6 years old 203 young adults below 25 years of age and 240 adults more than 25 years of age Every case present in year groups of adults is matched by 150 occurring in the toddler period

The problem of hemeralopia cannot be discussed here in all its aspects because the tropical features appear less specific and less important As regards the adults some attention should however be paid to epidemic nightblindness *Birnbacher (1927) after an intensive investigation of cases* in Austria stated that women between 20 and 50 years old suffering from nightblindness are never affected by xerosis The maximum incidence in males occurred at about age 19 in females generally between 10 and 15 years of age but in pregnant women between the ages of 20 and 30 The male fraction was 88 % Only rare instances of xerosis in pregnant women have been mentioned (Laffont *et al* 1950) After delivery hemeralopia ceases Among the Jogjakarta cases with xerophthalmia mentioned above there were only two pregnant women both suffering from liver disease For obvious reasons no accurate details can be given on nightblindness in toddlers but undoubtedly it is very often present Thus it appears that hemeralopia and xerophthalmia have a different epidemiology There are other arguments too for concluding that these anomalies though often coexisting depend on different mechanisms

F Incidence in Relation to Sex

Though less important from a health viewpoint the distribution of xerophthalmia as to sex is of consequence for a proper understanding Most authors agree that there is a preponderance of the male sex but opinion sometimes differs as to the age groups Tijssen (1936) claimed that kerato malacia was a disease of the first two years attacking the male and female sex equally in contradistinction to xerophthalmia later on in which there would be two boys to one girl He used the difference as an argument to explain the surplus of males in blind adult Indonesians Sie Boen Lian (1937) contested his opinion stressing that most of the infant cases died

whereas the older children in which the sex difference were affected to such a degree as to influence the sex r later life Sweet and K'ang (1935) denied the ex strength of probably biased figures and continuing their and Prescott (1953) conclude sex does not greatly quirements of vitamin A since in countries where xero mon both sexes are equally affected

To judge the complex but in part striking differe extensive material and a uniformity in diagnosis are req latter is specially inconvenient in relation to keratomala distinct manifestations in the young infant Of the 43 Blegvad (1924) 53 % were males 90 % of them under 116 cases of keratomalacia mentioned separately by M were boys which corresponds with the sex distributio 1511 cases of bakan 862 were boys representing 5 centage recurs so obstinately in large series of toddlers we believe it to be an essential feature of the condition large numbers of cases at a later age or in adult di cl fraction is considerably higher and presents an appre 85 % (Oomen 1957) The significance of the figures sh in Fig 30 Similar percentages have been reported fr Roels (Roels *et al* 1958) cases of Bitot spots in the Br were males Comparing the age incidence among boys Jakarta no significant difference as to the time et or covered the degree of severity in the affected appere While making a mental reservation for keratomalacia believe that *there are two definite attack rates caus d* a low one related to the toddler period resultin in every girl and a higher one affecting about 6 males to e the age of 10 that is before puberty

Sex difference with regard to serum levels of the vita and the liver reserves have been discussed several ti animal experiments (Moore 1957) but the predilection for the male sex in human beings did receive less atte sudden transition from the lower to the higher rate in t years is also evident in Fig 29 Tijssen (1940) C Oomen (1957) suggested that one of the causes of the h males in stillbirths and in infant mortality could be soug behaviour of the sexes as regards vitamin A metabolism for the higher rate in males has sometimes been attrib reasons The male child would have some preference ove

medical help is sought This at least is not true of the greater part of the data in the graph because for other causes the admittance rate was about equal Van Veen (Sagalaherang Report 1940) contended that the male child would get a smaller share of the carotene sources of the diet for

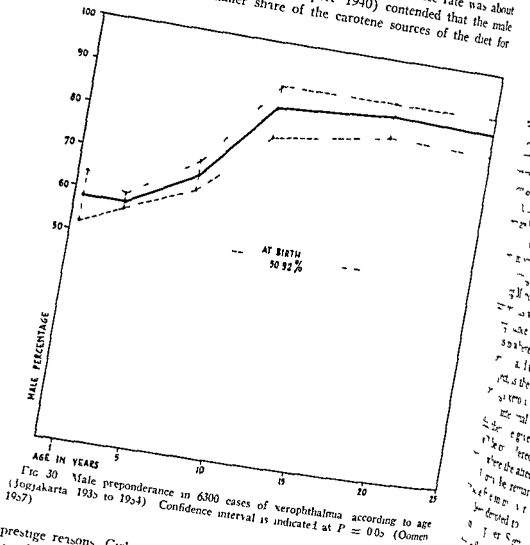


Fig 30 Male preponderance in 6300 cases of xerophthalmia according to age (Jogjakarta 1935 to 1954) Confidence interval is indicated at $P = 0.05$ (Oomen 1957)

prestige reasons Girls would have better access to the family pot Boys spend more time in the hot sun in work and play (Metivier 1941) It would be odd indeed if the aggregate of these trivial influences in many regions resulted in a constant proportion of 58 boys to 42 girls

There is little sense, as has occasionally been done in relation to kwashiorkor, in stating in health reports that xerophthalmia does not occur in the region, because potentially it can develop anywhere. The question is however whether the local customs or diets are such as to allow the condition to reach endemic proportions.

As to regional distribution, a few remarks can be made concerning Indonesia. In that country there is perhaps no hospital where the child stricken with xerophthalmia is not a regular or even daily visitor. Scores of cases occur in the large towns, even despite increasing care of the small child. Among the statistics of a single eye hospital, referred to frequently, in a heavily populated area of central Java, these cases represent about 3 % of the patients. Xerophthalmia has been reported from the rural highlands of North Sumatra (de Haas, 1931; Sie Boen Lian, 1937; van Manen, 1938; Gomperts, 1940), from plantations (Straub, 1927), from the lower regions of southern Sumatra (Maas, 1939) or Java (Oey Djoen Hoat, 1936), along the large rivers of Borneo (Hoogenkamp, 1956), from towns and coastal and mountain areas of Celebes (Oomen, 1954; van Wisselingh, personal communication, 1958). In none of the areas where formerly large nutrition surveys have been organized was it entirely absent, not even in prosperous rural areas (Patjet Report, 1940). The problem is present everywhere except perhaps on islands of eastern Indonesia where maize replaces rice as a staple food. Where comparison was possible coastal regions with better availability of fish showed a lesser incidence than the high valleys [Atchin, 5 and 8 % respectively, of xerosis and Bitot spots (Gomperts, 1940)]. A certain seasonal influence is noticeable, especially if a contrast exists between a wet and a dry (and meager) monsoon. In an accurate survey of school boys, a somewhat protected group, the incidence of Bitot spots varied from 0 to 4½ % (Klerks, 1956). Even if such percentages may not seem impressive, it should be repeated that 1 % of the graver lesions in the vulnerable age groups already constitutes a serious health problem.

VIII. Cure and Prevention

The reaction of the eye lesions to administration of vitamin A is so specific that a failure must cause serious reconsideration of the diagnosis. Still a number of controversial statements have been made; these are difficult to discuss, as they rarely indicate satisfactory evidence either on the nature of the affection or the condition of the patient. Vitamin A is apparently so rapidly and so completely absorbed from the intestine even under adverse conditions that evidence of the reverse suggests an abnormality. The most convincing argument for the diagnosis of a true

avitaminosis is the avidity with which the lacking nutrient is utilized There are in an individual patient such endless possibilities and the dietary history is usually so uncontrollable that one cannot be grateful enough for

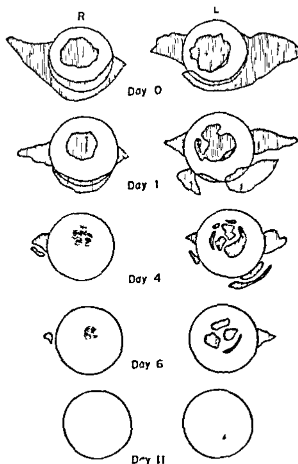


Fig. 31 Regression of xerosis conjunctivae et corneae in a Chinese adult during treatment. Areas affected on the day of admission and on the first, fourth sixth, and eleventh days thereafter (After Pillat, 1929)

the positive evidence In xerophthalmia the improvement of the ... tissues should be visible within 3 days (Fig 31)

Each xerotic spot of the cornea therefore should be considered to be emergency but with vitamin A repletion the treatment of the

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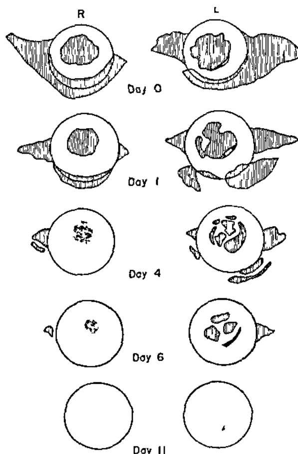


FIG 31 Regression of xerosis conjunctivae et corneae in a Chinese adult during treatment Areas affected on the day of admission and on the first fourth sixth and eleventh day thereafter (After Pillat 1929)

this positive evidence In xerophthalmia the improvement of the affected tissues should be visible within 5 days (Fig 31)

Each xerosis of the cornea therefore should be considered to be an emergency but with vitamin A repletion the treatment of the patient is

anything but finished His malnourished state should be treated according to the same principles as in kwashiorkor Even then the duty of the physician is not yet completed as xerophthalmia often is an environmental disease and it is essential to achieve improvement of the living conditions *The order of sequence of medical* actions should regard *first the eye second* the patient third his kitchen

As *in kwashiorkor* the safest approach to the impoverished tissues is via the normal physiology that is by administration *per os* and not parenterally *The mobilization of the tissue depots made by injection is apparently* slower and less reliable if immediate repletion is indicated On the other hand by the latter method *the physician knows for sure that the missing* nutrient is inside the body and may be mobilized during several days It is probable that even small *dose may perform the tissue repairs* and that serum levels are not the most reliable indicators of a cure In practice a combination of both approaches often will be unavoidable If manipulation of the eyelids is not objectionable instillation of vitamin eyedrops can be added but should never be regarded as sufficient in itself

If vitamin A for curative or preventive purpose is freely distributed among a less informed population high potency preparations should be avoided As normal fish liver oil is *effective* obviating the appearance of xerophthalmia and curing all except the severest forms this household item already popular in many tropical regions should be considered the remedy of choice The consumption of high potency preparations in capsules is less sure and often is not sufficiently *profitable at least not for the* patient Of course it still would be preferable if the diet contained regular *even though small quantities* of *liver eggs and milk As carotene for* the time being should be considered less reliable it should not receive too *much single emphasis in propaganda* but the importance of fat and protein should always be stressed simultaneously

It goes without saying that Mother and Child Care represents an extremely useful service to prevent and possibly cure the ill effects of xerophthalmia As the toddler represents the most vulnerable period such care should be extended until near school age

IX Approaches to and Recommendations Regarding the Xerophthalmia Problem

Before taking leave of his readers Moore (1957) after an impressive comprehensive review of the subject of vitamin A regrets that so many loose ends remain untied and so many clues have not yet been fully exploited From this review it is apparent how essentially meager is the

contribution from clinical sources and how important at least regionally the human problem

Here is a contrast as regards the position of thiamin which in the regions often mentioned here is of eminent health value Whereas in Indonesia by the understanding of the origin of the vitamin and by a few milling regulations beriberi has dwindled to a problem of minor proportions xerophthalmia continues to be as prevalent as ever in less accessible surroundings It is apparent that the solution will not be so simple for vitamin A as for B_1 The xerophthalmia problem is rooted deeper in diverse aspects of the popular diet and is more closely linked to prosperity A great deal of accurate investigation is called for before an adequate approach of the public health problem can be made A few suggestions may be given to delineate the work to be done

A TERMINOLOGY

1 A variety of terms has been used to describe the eye lesions due to vitamin A deficiency and the same term has sometimes been interpreted differently in different reports The terms prexerosis xerosis xerophthalmia keratomalacia Bitot spot pinguecula and pterygium should be clearly defined in all reports (Joint FAO WHO Expert Committee on Nutrition 1958)

2 It is suggested here that the term *xerophthalmia* be used collectively for all specific eye symptoms and not for any single one and *xerophthalmia syndrome* if alterations elsewhere in the body are implied

Xerosis conjunctivae (bulbaris) epithelialis is self explanatory

The aspect and the nature of the *Bitot spot* as a plaque produced by an abnormal secretion has been described

The terminological difficulty relates especially to the corneal affections The term *xerosis corneae epithelialis* is applicable to every case in which xerosis is demonstrable so long as the general structure is not yet affected Epithelial defects and small perforations do not alter essentially the nature of the affection A distinction could be made between *xerosis simplex* and *xerosis perforans*

Keratomalacia indicates a large scale necrosis with total destruction and softening of the cornea It is evidently followed by a phthisis bulbi A diffuse exsiccating necrosis of the whole cornea is to be called *mummification*

Prexerosis is a condition more easily distinguishable as a separate entity in the adult than in the small child In practice it would be advisable to relate it to the syndrome if the specific nature can be proved—for instance by the presence of typical symptoms including hemeralopia or by the specific reaction to administration of the vitamin

B Nutritional Pathology

3 The value of the Bitot spot as a symptom of xerophthalmia is beyond doubt The specificity of this phenomenon and its particular pathogenesis however still require a comparative study

The small differences in pathological processes which at one time produce only a localized perforation of the cornea and at another time a *malacia or a mummification* even simultaneously in the same patient are not yet understood satisfactorily

Both the clinician and the public health officer would benefit by a better insight in the nutritional pathology of the eye responsible for the development of polymorphic superficial keropathy (Djacos 1942) and of malnutrition keratoconjunctivitis (Blumenthal 1950) as a contrast to xerophthalmia The relation clinically and epidemiologically of hemeralopia and changes in the fundus oculi with xerophthalmia should be investigated

4 The significance of complete autopsies in cases of xerophthalmia both of the slighter and of the severe affections cannot be sufficiently stressed

5 The value of carotene as a precursor of axerophtol in the human body should be reconsidered under the conditions and in the regions where xerophthalmia is endemic The influence especially of the fat and protein moieties in the respective diets particularly the rice diet with emphasis on the intake of the young child should be fully investigated

6 Approaches should be sought to determine the reserves of vitamin A in expectant and lactating mothers and in newborn infants The variations in vitamin A intake either caused by the deficient content in breast milk or by a decreased intake should be studied

7 The influence of various infections and infestations on the absorption or availability of vitamin A deserves further investigation

8 The possibilities of congenital or constitutional variations in relation to the development of xerophthalmia require clinical attention Metabolic differences between cases of kwashiorkor with and without xerophthalmia respectively merit closer attention (excretion of the vitamin destruction by red blood cells etc)

9 The sex distribution especially in cases occurring in the first year and in keratomalacia should be further investigated The nature of the difference in the low age and the adult sex rate in xerophthalmia still has to be explained The low age sex rate suggests a relation of vitamin A with the preponderance of the male sex in infant mortality

10 Particularly promising it would seem is a comparative study on the occurrence of xerophthalmia in tropical regions where red palm oil is

an essential part of the diet and in regions where carotene is consumed in other forms

C Public Health Measures

11 In view of the serious significance of the occurrence of xerophthalmia it should be made compulsorily reportable in regions where its prevalence demonstrably influences the problems of blindness or of child mortality

12 Because of the close relation to protein malnutrition and in view of the serious consequences of either condition it is imperative that in such cases the eyes always be fully examined their condition noted in reports of protein malnutrition and the significance of the terms used to describe the affection of the eyes clearly explained (McLaren 1958)

13 In view of prevention and cure it would seem desirable to develop simple measures and to assess the minimal dose of vitamin A that can protect a child during the vulnerable period The nature of relapses after vitamin A therapy should be investigated

14 The knowledge of symptoms and treatment of xerophthalmia should be promoted especially where the population has not regular access to trained medical help

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Immunochemical Staining with Fluorescent Antibody

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I Introduction

At the present time an article on fluorescent antibody research for a review of tropical medicine must be justified more on the basis of potential achievement than on grounds of past accomplishment Although much work with this technique in the field of infectious diseases is as applicable to medicine in the Tropics as it is to medicine in temperate zones tropical medicine in the more restricted sense has as yet barely felt the impact of this ingenious and extremely useful tool Until recently technical complexities of the procedure served to restrict its use to rather well equipped research laboratories At the present time however the technique has become simplified to the extent that almost any laboratory which uses a microscope can undertake work with this tool The limiting factor now is more likely to be availability of well trained personnel since in common with other fine tools this one is best employed by craftsmen

A thorough review of techniques and findings up to that time was published in 1956 by A H Coons who is mainly responsible for introducing and developing this procedure In 1958 another review appeared by the same author primarily concerned with techniques Since these articles were published significant progress has been made in simplifying the labeling of antibodies in making available new dyes of different colors

that may be employed for this purpose and in applying the technique to the solution of practical diagnostic problems In addition to describing these new developments in some detail the present review will attempt to demonstrate the versatility of the fluorescent antibody method by referring to literature published since Dr Coons reviews appeared Studies of tissue pathology and pathogenesis will not be considered here unless they demonstrate technical principles that may be more generally useful An appendix containing important sources of reagents and equipment is to be found at the end of this article

In staining with labeled antibody the immunologist undertakes to demonstrate on the cellular level serologic reactions which are generally studied in the test tube The theoretical framework for the method is simple Antiserum developed in experimental animals or in man is conjugated by mild chemical procedures with any of several fluorescent dyes If a drop of fluorescent antiserum is applied to a smear or section containing homologous antigen precipitates form at the antigenic sites After such preparations are rinsed to remove unreacted conjugate the precipitates can be seen *in loco* with a fluorescence microscope The advantage of using fluorescent compounds as labels over nonfluorescent highly colored dyes is that much smaller quantities of the former can be detected microscopically

II Technical Considerations

A Microscopy

A thorough review of fluorescence microscopy in general plus a complete bibliography and list of sources of equipment are given by Price and Schwartz (1956) A discussion limited more specifically to fluorescent antibody requirements and supplies is to be found in a manual by Cherry *et al* (1959) For purposes of the present review only the following points are emphasized (1) standard microscopes equipped with glass (as opposed to quartz) optics are perfectly satisfactory (2) high intensity light sources rich in blue and near ultraviolet wavelengths are necessary (3) glass and gelatin color filters can be used to isolate the wavelengths needed and (4) fully equipped packaged units are available commercially from several manufacturers

B Labeling Agents and Procedures

1 *Green Fluorescing Dyes*

Most of the literature in this field has been based on use of proteins labeled with fluorescein isocyanate (Coons *et al* 1942 Coons and Kaplan 1950) This relatively unstable compound was initially employed as an

acetone solution immediately after its synthesis from the last stable intermediate aminofluorescein Conversion of the amino compound to isocyanate requires phosgene Subsequently it was reported that the isocyanate could be preserved in dry acetone solution for from several weeks to a year depending upon the temperature at which it was stored (Marshall 1951 Coons 1956) Goldman and Carver (1957) found that fluorescein isocyanate could be stabilized by drying solutions onto thick filter paper which could be stored for at least 7 months in a desiccator at room temperature The addition of strips of paper containing appropriate amounts of isocyanate to buffered globulin resulted in conjugation In contrast to labeling procedures employing solutions of dye little or no denaturation of protein occurred with the filter paper method

The most significant improvement in use of fluorescein as a protein label was the synthesis by Riggs *et al* (1958) of the isothiocyanate derivative This reagent prepared by reacting aminofluorescein diacetate (Riggs *et al* 1958) or aminofluorescein (Marshall *et al* 1958) with liquid thiophosgene instead of with gaseous phosgene is a stable powder which is available commercially Marshall *et al* compared staining titers obtained with conjugates prepared by two methods employing isocyanate and three methods employing isothiocyanate Highest titers were obtained with isothiocyanate dried on filter paper or added as a powder to buffered antisera Because of its simplicity and effectiveness the latter procedure was recommended as the method of choice

2 *Yellow Fluorescing Dyes*

Weber (1952) described physical and chemical characteristics of albumins conjugated with 1 dimethylaminonaphthalene 5 sulfonyl chloride (DNS) The dye was mentioned briefly by Clayton (1954) as one of the labels employed with antisera against embryonic tissue Preparation and use of the compound as an immunochemical stain were presented in considerable detail by Mayersbach (1958) and concurrently in lesser detail by Redetzki (1958) Dry sulfonyl chloride which is available commercially is stable for several months when kept in a desiccator at 4–7 C It reacts with free amino groups on proteins to form sulfonamido bonds Conjugated proteins are stable and the dye resists dissociation during precipitation of conjugates with alcohol acetone or ammonium sulfate

Labeling is accomplished by adding an acetone solution of dye to chilled protein buffered at pH 7 5–8 0 Unreacted undissolved dye is removed by centrifugation unreacted dissolved dye is dialyzed away or removed by precipitating the protein with ethanol or ammonium sulfate When DNS is added to protein in the proportion of about 6% by weight the number

of dye groups introduced per molecule of gamma globulin is approximately four

Antisera labeled with DNS retain their original immunochemical characteristics unimpaired Although the free dye is considerably less fluorescent than free fluorescein conjugated proteins do not show such disparity (Mayersbach 1958) Similarly localized antigen is comparable in fluorescence when treated with either DNS or fluorescein

To the author's knowledge there have been no further reports in the literature of the use of antibodies labeled with this compound for staining purposes In view of the over all excellence of the yellow green fluorescein tag and the simplicity of labeling with the isothiocyanate derivative of fluorescein a yellow fluorescent dye does not offer as many advantages for contrast staining as the orange red fluorochromes described in the next section

3 *Orange red Fluorescing Dyes*

The first use of a red fluorescing label was by Clayton (1954) who coupled nuclear fast red (described as benzaldehyde 6-nitro 2 sodium diazotate) to antisera Unfortunately the formula given in the publication does not correspond to the dye actually employed (Clayton personal communication 1955) Use of nuclear fast red is as follows (Clayton personal communication 1955) The fluorescent component of the dye is removed with alcohol water on a starch column dissolved in acetone or water and added dropwise to ice cold serum (neutral or unbuffered) to a maximum of 10% by volume The uncombined dye is removed by repeated precipitations with ammonium sulfate or by electrolysis through a dialyzing sac It will not dialyze unaided Since no other work has appeared in which this dye was employed as a protein label its usefulness is difficult to estimate

Four rhodamine derivaties fluorescing in orange and red have been used as antibody labels rhodamine B isocyanate (Silverstein 1957) tetramethylrhodamine isocyanate (Hiramoto *et al* 1958) rhodamine B isothiocyanate (Riggs *et al* 1958) and lissamine rhodamine B 200 (Chadwick *et al* 1958a b) The two isocyanates were employed in the same manner as the corresponding fluorescein derivative Although no information was given as to stability of the dyes it may be assumed by analogy to fluorescein that they are relatively unstable compounds They are not marketed commercially and are not likely to enjoy much popularity in view of the availability of the other two rhodamine derivatives

Rhodamine B isothiocyanate is similar to fluorescein isothiocyanate in being a stable powder which is available commercially It is employed in

the same manner to label proteins and has been shown by its developers to be a useful tag for specific staining of *Bacillus anthracis Pasteurella tularensis Rickettsia typhi* and adenovirus RI 67

Lissamine rhodamine B 200 (RB 200) was the best of nine fluoro chromes studied by Chadwick *et al* (1958a) for possible use as protein labels The stable commercially available dye must be converted to the sulfonyl chloride derivative before being coupled to protein through the sulfonamido linkage The dye powder is ground in a mortar with PCl and the mixture which contains the sulfonyl chloride is extracted with dry acetone The acetone solution is added dropwise to chilled buffered protein at pH 9 and allowed to react for about 30 minutes Uncombined dye is removable either by dialysis against saline or by sorption with activated charcoal When the proportion of dye to protein is 4% by weight three molecules of dye are introduced per molecule of albumin

Fluorescence intensity of preparations stained with rhodamine labeled antibodies is reported to be comparable to that of fluorescein The distinct color contrast between the two dyes and the contrast between the color of rhodamine and the blue autofluorescence of most tissues make rhoda mine an excellent complement to or substitute for fluorescein This may be particularly true in staining arthropods such as ticks whose exo skeleton fluoresces in the same color as fluorescein (Goldwasser and Shepard personal communication 1959) Lissamine rhodamine B 200 can be purchased cheaply but the necessity of converting it to the sulfonyl chloride is a deterrent to its use in comparison with rhodamine isothiocyanate At the present time the stability of the acetone solution of RB 200 sulfonyl chloride is not known although successful conjugations have been achieved up to 18 hours after preparation (Chadwick *et al* 1958b)

4 Other Dyes

Recently Hess and Pearse (1959) recommended a group of cellulose reactive dyes as protein labels These dyes bear a dichlorotriazinyl group ing and are coupled through free amino groups on the proteins On the basis of reported preliminary results the possibility exists that a whole new group of dyes may become available for antibody tagging

C STAINING TECHNIQUES

In the procedures described below results must always be evaluated by comparison with the amount of staining that occurs in control prepara tions Tissue cells and amebas tend to take up considerable protein non specifically although this can be reduced by sorbing labeled solutions

with tissue powders before use (Coons *et al* 1955) Bacteria on the other hand, are singularly free of this tendency so that it is not necessary to sorb the reagents when staining such material However each new antigen antibody system should be evaluated separately for evidence of nonspecific reactions

1 Direct Staining

The basic reaction in fluorescent antibody procedures is that which occurs between labeled antibody and unlabeled antigen to produce a labeled product (Figure 1)



Fig 1 Direct staining procedure

2 Indirect Staining

A procedure commonly used in staining viruses and in studying pathology caused by autoantibodies and other soluble agents was reported by Weller and Coons (1954) In this method an additional step is introduced in the above procedure Unlabeled antibody is first reacted with unlabeled antigen The bound antibody, a gamma globulin is then demonstrated by staining it with labeled antibody (prepared in a different species) directed against gamma globulin thus indirectly identifying the original antigen under investigation (Fig 2)





Fig 2 Indirect staining procedure

One advantage of the indirect procedure is that a single labeled antibody e g antirabbit prepared in a goat can be used to stain any antigen for which one may possess antiserum made in rabbits As a result of the recent simplification in labeling procedures however this advantage has

lost some of its original importance Since in any case specific antisera need to be prepared in order to stain the antigens under investigation it may be just as simple to label each one separately for use in the direct procedure

The indirect method offers a more important advantage for antigen antibody systems in which direct staining does not produce adequate brightness In such cases the layering effect of the indirect method may increase significantly the sensitivity with which one can detect the antigen being studied (Liu 1957 Kaplan 1958)

The indirect procedure has also served as the basis for serologic tests for the presence of antibody in unknown sera (Liu 1957 O dea and Dineen 1957 Deacon *et al* 1957) By employing known antigen obtained from cultures or other sources and labeled antiserum directed against say human globulin it is possible to determine whether a given human serum possesses antibodies to the antigen under consideration A dilution of unknown unlabeled serum may be applied to the known antigen as in step 1 of Fig 2 After brief rinsing the labeled antibody against human serum is applied If the antigen becomes fluorescent following these manipulations it indicates the presence of antibody in the unknown serum Suitable positive and negative controls are necessary for proper interpretation of the observed results

A disadvantage of the indirect procedure is that it multiplies the number of controls needed to prove the specificity of the reaction since nonspecific reactions may occur in both steps of the procedure When the method is used as a serologic test one is limited to testing sera only from the species for which one has labeled antisera In dealing with an organism with a wide host range e g *Toxoplasma gondii* this restriction limits the testing that can be performed of possible animal hosts A third disadvantage is that the two step procedure is relatively cumbersome if one wishes to perform large numbers of serologies on a routine basis

3 *Complement Staining*

This variation of the indirect procedure is based upon the fact that bound complement may be stained by using labeled antiserum directed against the complement source rather than against the specific antibody (Goldwasser and Shepard 1958) For example tissue culture cells infected with poliovirus were exposed to a mixture of immune human serum plus fresh normal guinea pig serum as a source of complement After the usual incubation and washing labeled rabbit antibody prepared against guinea pig globulin was applied Bright staining of the virus was obtained This method seems applicable to any antigen antibody system

that will bind complement Its main advantage aside from the possibilities it offers for the study of complement is that only one labeled antiserum against guinea pig globulin may be used to stain many systems regardless of the source of specific antibodies

As mentioned above for the indirect method the ability to stain many antigens with a single labeled antiserum has lost some of its importance with the simplification of labeling procedures Use of an additional reagent complement entails further controls to assure specificity of results In this regard Goldwasser and Shepard (1958) reported that some normal guinea pig and monkey sera when employed as sources of complement caused staining in the absence of immune serum On the other hand by employing labeled immune serum plus labeled anticomplement serum it may be possible to increase the brightness of otherwise weak staining reactions Detection of antibody in unknown sera by complement staining is similar to that described for the indirect staining method with the added advantage that serum from many species may be tested with the same labeled anticomplement serum

4 *Inhibition Procedure*

A control frequently used to demonstrate specificity of the direct staining reaction is to inhibit staining by first exposing the antigen to unlabeled antibody (Coons and Kaplan 1950) This has the effect of blocking further reactions of the antigen resulting in reduced or inhibited fluorescence A modification of this reaction has been developed as a serologic test for antibodies in unknown unlabeled sera (Goldman 1957b) The test is performed as follows A dilution of unknown test serum is added to an equal amount of an appropriate dilution of labeled known antiserum The mixture is applied to a smear or section containing known antigen and the slide is examined after the usual incubation rinsing and mounting procedures In this case *absence* or *reduction* of fluorescence indicates a positive test for antibodies

This procedure has the advantages that serum from any animal species can be tested with the same labeled antibody solution and that the mechanics of the test are simple since only a single application of reagent is necessary For incompletely understood reasons the two step inhibition procedure which was described originally (Coons and Kaplan 1950) and which has been widely used with many systems is not always successful with bacteria and other forms (Moody *et al* 1956 Goldman 1957b Poetschke *et al* 1957 Levina 1958) Moody *et al* (1956) and Goldman (1957b) reported better results using the one step procedure

E Manipulation of Slides

Staining manipulations consist of adding a few drops of antibody solution to the section or smear allowing the reaction to take place in a moist chamber (generally for 30 minutes to 1 hour), rinsing the preparation and mounting it with slightly alkaline glycerol Staining periods extending for several days have been reported (Finck *et al* 1956) but this is uncommon

In order to obtain reproducible results particularly when performing serologic tests it is advisable to standardize these manipulations The general availability of 37 C incubators makes this a convenient temperature at which to run reactions Moist chambers can be made for small numbers of slides by placing wet filter paper under a petri dish cover and for larger numbers by using a desiccator jar with water instead of desiccant under the porcelain plate Deacon *et al* (1957) reacted smears on a rotating machine and obtained more satisfactory results than with immobile slides When several smears are stained on a single slide shallow cells for limiting applied solutions may be formed conveniently with nonaqueous marking compounds such as Marktex Tech Pen ink Semipermanent mounts can be made by sealing glycerol preparations with cosmetic nail polish In this laboratory, fluorescent amebas have shown no loss of fluorescence for weeks when mounted in glycerol and stored at —70 C Smears kept at —20 C and 5°C have not held up as well when fluorescence was measured quantitatively but in qualitative evaluations the reduction in fluorescence has not been significant over periods of weeks even at 5 C Preparations may be mounted in more nearly permanent media with xylol soluble Fluormount from Edward Gurr Ltd (Chadwick *et al* 1958a)

F Examination of Preparations

It is well to realize that continuous exposure of fluorescein stained preparations to light from the microscope illuminator may result in significant fading of stained organisms Goldman (1960) showed that fluorescence of fluorescein solutions may drop as much as 20–30% within 30 seconds depending upon intensity of the light and brightness of initial fluorescence In evaluating the staining capacity of conjugates in reading slides for serologic testing or in any comparative testing fading due to bright illumination may confuse results unless one is cognizant of the extent of the phenomenon Although rate of fading is higher with more intense light and with higher fluorescein concentrations absolute brightness is greatest under these same conditions For this reason greatest sensitivity can still be attained with bright light sources and with highly active conjugates

In recording fluorescence photographically one result of the fading phenomenon is to make short exposures of bright objects preferable to long exposures of duller subjects Since background fluorescence may not fade to the same degree as fluorescein if at all longer exposures may result in reduced contrast in the negatives With films like Eastman Tri X or Ansco Super Anscochrome exposures of 1–3 minutes have been suitable in our laboratory for bacteria viruses and protozoans at magnification of 1000 ×

G Quantitation of Fluorescence

In the earlier localization studies with fluorescent antibody qualitative findings were of prime importance and little attention was paid to objective quantitation With extension of the method to diagnostic and analytic problems objective quantitation of fluorescence intensity has become more important

Thomason *et al* (1959) compared staining results by computing numerical figures from a formula embodying percentage of stained cells type of staining and a subjective estimate of intensity of fluorescence In the absence of complex light measuring equipment relative quantitation is still possible based on ordinary dilution and titration procedures Notations involving plus marks to indicate intensity of fluorescence have little meaning from one laboratory to another but within the same laboratory such estimates are of considerable value

Microfluorimetric determinations of fluorescent antibody reactions have hardly been attempted Mellors *et al* (1955) photographed individual microscopic fields showing glomeruli and kidney tubules After the use of standardized procedures for developing the films they compared the density of various portions of the field with a microdensitometer This approach had the advantage that fluctuations in the light source mechanical and optical misalignments and other influences were canceled out since the same field was being compared internally A serious limitation on the method is that exposure periods of 5 minutes were used Since fading was probably greater for the initially brighter portions of the field than for the less bright portions the initial and true contrast was probably reduced Finally recording the brightness of individual organisms in smears would be too slow with presently available film to allow extensive quantitative studies

In the author s laboratory a relatively simple microfluorimeter has been constructed which measures directly the light flux from fluorescent cells (Goldman 1960) With this instrument it is possible to obtain objective

values of light intensity in terms of photomultiplier anode current under controlled and specified conditions Conversion of measurements into absolute values of fluorescein concentration (and thus indirectly to antibody concentration) depends primarily on establishing valid calibration curves for fluorescein bound to protein and fixed in space on a cellular substrate This represents a considerable technical problem since small changes in molecular environment may have profound effects on fluorescence emission (Ornstein *et al* 1957) and since suitable models for fluorescent microorganisms have not as yet been proposed

Microfluorimeters have been described in which scanning and recording of fluorescent exfoliated cells were automatic (Mellors and Silver 1951) and in which fluorescence spectra were obtained of benzpyrene stained sections (Norden 1953) Instrumentation from commercial sources is easily sensitive enough to measure the fluorescence of a single bacterial cell stained with fluorescent antibody although as with all highly sentitive techniques methodological problems and pitfalls abound Since the greatest value of labeled antibody procedures is not likely to be achieved without some form of objective quantitation further investigations into techniques of microfluorimetry are highly desirable

III Applications of Fluorescent Antibody Methods

A Viruses and Rickettsia

Because of the small size of these agents and the difficulties of visualizing them with conventional staining procedures fluorescent antibody studies in this area have tended to emphasize distribution and fine localization of agents in tissue cultures and in infected animals The considerable success of this type of study has more recently stimulated an interest in specific diagnosis based upon demonstration of etiologic agents in lesions or in isolates from patients Serologic procedures based upon fluorescent antibody have also been explored

Development in tissue cultures of agents responsible for psittacosis (Buckley *et al* 1955) measles (Cohen *et al* 1955) vaccinia (Noyes and Watson 1955) herpes simplex (Lebrun 1956) poliomyelitis types 1 2 and 3 (Buckley 1956 1957) fowl plague (Breitenfeld and Schafer 1957) and type 4 adenovirus (Boyer *et al* 1959) have been reported Close correlation or identity between structures visible by conventional microscopy and areas stained specifically with labeled antibody was a characteristic finding except in the case of herpes simplex virus Type A inclusion bodies characteristic of the latter agent were found to represent an intranuclear scar following the shift of viral antigen from nucleus to cytoplasm In the case of fowl plague virus which has been shown to

consist of two distinct antigens the intracellular distribution of each was demonstrated by employing specific antisera against the two antigens separately and against the whole virus

Localization of neurotropic Egypt 101 virus in infected mice was studied by Noyes (1955) Virus antigen was found in neurons of infected mouse brain and spinal cord Tissue cultures of a human epidermoid carcinoma to which the virus was finally adapted also showed intense staining

Distribution of canine distemper virus has been investigated in natural infections of dogs (Moulton 1956 Coffin and Liu 1957) and in experimental infections in ferrets (Liu and Coffin 1957) Moulton observed stainable virus in astrocytic and possibly other glial nuclei in the cerebellar white matter Myelin sheaths neurons and axis cylinders showed no specific fluorescence in spite of the fact that demyelination is a characteristic finding in central nervous system lesions that occur in this infection Coffin and Liu found antigen in visceral and cutaneous lesions as well as in the brain depending upon the stage of infection Contrary to Moulton s findings antigen was observed in neuron cell bodies dendrites and axons as well as in ependymal cells and astrocytes Moulton used 15 μ thick frozen sections compared to the 4–6-μ sections studied by Coffin and Liu In addition the latter workers fixed their sections with acetone whereas Moulton stained his dried sections without previous fixation Whether these technical differences are sufficient to account for the reported differences in virus distribution was not determined

In ferrets where distemper runs a fulminating fatal course Liu and Coffin found viral antigen in the cervical lymph nodes 2 days after intranasal inoculation Viremia occurred on the third and fourth days following infection virus spreading through the reticuloendothelial system and thence generally throughout the tissues Labeled antibody was much more sensitive than conventional pathologic methods for detecting evidence of infection in cells Peripheral blood smears showed specific antigen in leucocytes as early as 3 days after infection before fever and clinical symptoms developed and remained positive for virus until the animals died

Cellular localization of Shope papilloma virus in natural and experimental papillomas of rabbits was studied by Noyes and Mellors (1957) Brilliant fluorescence was demonstrable in nuclei of the keratohyaline and keratinized layers only Antigen could not be demonstrated in deeper proliferating cells of the papillomas The authors postulated that virus might exist in nuclei of deeper cells as a nonantigenic nucleic acid moiety lacking protein Stainable antigen might then represent a later develop

mental stage of the virus when antigenic protein became associated with the nucleic acid Subsequently it was shown by micromanipulative techniques that infectious virus was limited to the same areas that were stained by specific antibody (Noyes 1959)

The possibility that virus particles might exist in a form in which they were not demonstrable by labeled antibody was suggested also by work with Newcastle disease virus (Prince and Ginsberg 1957) When Ehrlich ascites tumor cells were incubated with virus and inoculated into mice virus was first demonstrated on the cell surfaces a latent period then occurred in which virus antigen was not stainable Finally fluorescent antigen appeared in the cytoplasm of the infected cells

Diagnostic applications of fluorescent antibody to virus infections may be dated from the attempt by Liu (1956) to identify influenza in nasal washings from patients with acute respiratory illness Smears of cellular sediment obtained in washings were stained with labeled rabbit antiglobulin to influenza types A and B Specific fluorescence in smears was taken to indicate viral infection Results of fluorescence examinations were compared with results of hemagglutination inhibition tests performed with acute and convalescent sera from the same individuals Twelve out of 17 cases (71%) of influenza A prime and 10 out of 26 cases (38%) of influenza B were correctly diagnosed with fluorescent antibody One false positive occurred among 23 noninfluenzal patients Although diagnosis by staining was obviously less sensitive than by conventional serology the former results were available the day samples were taken compared to the 10–14 days needed for hemagglutination inhibition

These results demonstrate some of the virtues and limitations of etiologic diagnosis with labeled antibody In the presence of sufficient antigen specific staining is faster than and as reliable as more conventional procedures provided that a sound immunological foundation has been laid previously for the agent under study On the other hand direct microscopic examination may not be sensitive enough to detect very small numbers of stainable particles In Liu s work apparently no effort was made to stain tissue cultures after inoculation with nasal washings The possible enrichment of virus material by cultivation for short periods might have increased the number of positives detected since in 2 type A prime cases positive cultures were obtained from washings which were negative by immediate staining

Rapid diagnosis of distemper in febrile dogs by direct staining of conjunctival smears was reported to be a simple and practical approach particularly in early stages of the disease when clinical diagnosis is difficult (Coffin and Liu 1957) In autopsy material smears of urinary

bladder mucosa or dab smears from lungs were useful for detecting systemic infections

Goldwasser and Kissling (1958) showed that Negri bodies in brain smears from rabies infected mice were stainable with labeled antibody More useful from a diagnostic standpoint was their finding that infected brains that were negative for Negri bodies also showed antigenic particles and clusters In addition they were able to demonstrate rabies virus in salivary gland smears of infected wild and domestic animals

Rapid diagnosis of poliomyelitis virus by specific staining of monkey kidney tissue cultures was attempted by Kalter *et al* (1959) The method was to stain smears of Versenized cells from tissue cultures showing a 1 to 2+ cytopathic effect following inoculation with suspected stool specimens Labeled monkey antipoliovirus serum was used as the specific staining agent In a series of 50 stools fluorescent staining results agreed with conventional isolation and neutralization tests in 47 cases Of 33 polioviruses 30 were correctly typed 3 were missed by the staining method Seventeen stools containing Coxsackie ECHO or no entero viruses were negative by staining Staining results were obtained within a few hours of the time that the cytopathic effect was observed and considerably earlier than by conventional methods

Herpes simplex infections were diagnosed by direct staining of scrapings from vesicular lesions and from one nasal smear (Biegeleisen *et al* 1959) In 15 specimens studied by staining and by chick embryo isolation the same 8 specimens were found positive for virus by both methods No discordant results were obtained Inasmuch as staining could be performed immediately whereas viral isolation required 3 days the value of specific staining for rapid diagnosis was obvious

Rickettsiae of Rocky Mountain spotted fever have been demonstrated in smears made from ticks collected in the field (Goldwasser and Shepard personal communication 1959) Of 140 ticks collected in an area with known incidence of spotted fever 4 were positive by staining with fluorescent antibody and the same 4 were positive by yolk sac inoculation All others were negative by both methods Of 91 ticks collected in an area free of this infection none were positive either by staining or by isolation methods

Employment of the indirect staining procedure as a serologic test for antibodies to various agents has also been reported O dea and Dineen (1957) found herpes simplex antibodies in 5 out of 8 random human sera correlating precisely with virus neutralization tests performed in eggs They used as antigen tissue cultures of human amnion and infant mouse kidney cells infected with two strains of herpes simplex virus

Liu (1957) demonstrated the presence of antibody to primary atypical pneumonia (PAP) in rabbit sera by using sections of infected chick embryo lung as antigen Subsequently more extended observations on human infections were reported (Liu *et al* 1959) In spite of the fact that the nature of the antigen (4–6 μ thick frozen sections of infected lung usable for 2 weeks when stored at 4 C) militates against widespread employment of the test the procedure is of interest in being the first specific serologic test for primary atypical pneumonia All of 8 available strains of PAP virus were suitable as antigen and no cross reactions occurred with influenza psittacosis Q fever adenovirus and pneumonia virus of mice Out of a total of 89 patients studied in groups during various outbreaks of clinical PAP infection from 67 to 90% were serologically positive by fluorescent staining depending upon the group involved

Ordinary complement fixation procedures do not distinguish between antibodies to the two closely related rickettsial species responsible for murine and epidemic typhus unless sorption procedures are used In a serologic method proposed by Goldwasser and Shepard (1959) anti serum dilutions were prepared in soluble type antigens extracted from both species and also from uninfected yolk sacs The antiserum antigen mixtures were layered onto smears prepared previously from murine and epidemic typhus rickettsiae Following a brief incubation period the overlay was rinsed off and labeled antihuman globulin was applied to each smear as in the usual indirect staining procedure In most cases staining results agreed with those to be expected on the basis of classic sorption methods i e serum diluted in homologous antigen no longer caused staining of either homologous or heterologous rickettsiae but serum diluted in heterologous antigen retained its affinity for the homolo gous species while losing it for the heterologous species This unusual approach to sorption procedures may be useful in other systems as well since only small amounts of reagents are necessary and manipulations are simple and brief

B Bacteria Fungi and Spirochetes

In this group of agents whose size is such that they are readily visible with conventional microscopy fluorescent antibody studies have empha sized specific identification and differentiation of morphologically similar species In addition antibodies in human sera have been detected with smears of intact organisms as antigen

Moody *et al* (1956) working with *Malleomyces pseudomallei* showed that a 15 minute exposure to labeled antibody stained dried smears of the

homologous species brilliantly and distinctively No cross reactions occurred with 20 strains of other bacterial species cross reactions did occur with the closely related species *M mallei*

The remarkable sensitivity of the staining procedure was shown in studies of pure cultures and of mixtures (Thomason *et al* 1956) *M pseudomallei* was detectable in suspensions containing as few as 220 homologous cells per milliliter regardless of whether suspensions were prepared from pure cultures or whether they contained massive numbers of other bacteria In experimentally seeded soil the organism was detectable in samples containing 2×10^4 cells per gram of soil Fluorescent cells were recovered from surfaces exposed to aerosols containing $1\ 6 \times 10^4$ cells per cubic foot In all cases direct examinations were made of the involved materials without enrichment by cultivation

Hobson and Mann (1957) prepared labeled antisera against bacteria isolated from rumen contents of cattle They were able to stain streptococci gram negative cocci lactobacilli and gram negative rods in rumen contents of sheep and calves Although no extensive serologic and cultural cross checking was performed it was clear from the preliminary work that this method of locating and identifying organisms directly from the gut contents was feasible

A retrospective study of an epidemic of diarrhea caused by enteropathogenic *Escherichia coli* was performed by Whitaker *et al* (1957) Working with 128 frozen stools obtained three years previously direct smears were stained with type specific (0127 B8) anti *E coli* labeled globulin Staining with fluorescent antibody confirmed as positive all 53 specimens originally diagnosed by conventional rectal swab techniques In addition thirty nine individuals who had been positive by stool cultures at some time during the epidemic (but not at the time the frozen specimens were collected) were also found positive by staining The clinical and therapeutic history of these cases suggested that the staining technique had revealed nonviable organisms which had been missed originally by rectal swab cultures Specimens from three patients currently hospitalized for diarrhea were diagnosed as containing *E coli* 0127 B8 by direct staining and subsequently by cultural and agglutination methods Specimens from twenty other patients negative for this strain by conventional methods were also negative by staining This important contribution demonstrated (1) that clinical material could be stained directly for species and strain identification of bacteria (2) that organisms which were nonculturable by reason of chemotherapy or physical manipulations could still be stained specifically with appropriate labeled antibody

and (3) that one could identify this group of bacteria more quickly and easily by the staining procedure than by conventional methods

De Repentigny and Frappier (1956) prepared labeled antiglobulin against aqueous washings of *Hemophilus pertussis* grown in liquid medium. Homologous bacterial smears made from liquid or solid culture media stained brightly with this reagent demonstrating for the first time the presence of an antigenic capsular layer in this species even when grown in liquid medium

Moody *et al* (1958) employed suitably sorbed antisera to stain streptococcus groups A B C D F and G in dried smears of pure cultures After this they compared results of staining procedures and conventional grouping methods on throat swab material obtained from eight patients In four cases group A streptococci were found by both methods in three cases no group A was found by either method and one case was doubtful by staining and negative by culture The significance of these results for the diagnostic laboratory lies in the fact that the staining procedure yielded results in from 30 minutes to 6 hours compared to the 3–5 days needed for conventional procedures

Halperen *et al* (1958) studied staining reactions of labeled antiglobulin to group A streptococci In common with Moody *et al* (1958) they found a degree of cross reaction with group C but none with groups D and G Five other bacterial species commonly found in throat smears were mixed with streptococci on slides and found not to stain with the anti group A globulin Direct throat smears were stained from 49 patients with acute pharyngitis from whom β hemolytic streptococci were isolated Out of 33 positive by culture for groups A and C 30 (91%) were also positive by staining Of 50 throat smears from patients with upper respiratory infections who were negative for β hemolytic streptococci none was positive by staining

Thomason *et al* (1957) prepared separate antisera against each of the three classes of antigens found in *Salmonella typhosa*—somatic flagellar and envelope—and against all three antigens together Portions of each antiserum were labeled and employed to stain dried smears of bacteria Unlabeled portions of the same antisera were used in slide agglutination tests with the same cell suspensions used for staining It was found that each of the three antigenic components in the intact bacterial cell could be stained specifically and exclusively by homologous antiglobulin or all three components could be stained simultaneously with appropriate anti body Specificity of staining was equivalent to that shown by agglutination tests taking into consideration the different physical conditions of the two reactions For example flagellar agglutination sometimes succeeded

in the absence of specific staining because of loss of flagella by physical manipulations These results showed that visible staining obtained with labeled antibodies reflected classic serologic reactions of this group as demonstrated by agglutination and sorption procedures

In further studies with *Salmonella* Thomason *et al* (1959) investigated the possible usefulness of fluorescent staining for identifying this group in fecal specimens or rectal swabs Two labeled polyvalent antiglobulins were prepared to 45 and 8 different 0 groups of *Salmonella* respectively to be used as screening reagents and to *Salmonella typhosa* and *Vibrio comma* somatic antigens for further checking of positive stool specimens The globulins were first evaluated against pure cultures of various enteric bacteria In general there was excellent agreement between known serologic relationships of the strains tested and staining reactions However when rectal swabs or stool specimens were examined with labeled antibody the multiplicity of serologic cross relationships with *Proteus Escherichia* and *Aerobacter* (as determined by cultural and serologic studies on the same specimens) made it impossible to use the staining reaction for pinpointing infections with *Salmonella* alone Thus in this case the sensitivity of the staining method proved to be a handicap rather than an aid to diagnosis

Poetschke *et al* (1957) studied antigenic relationships of the bacterial phase of *Proteus morgagnii* and its labile and stable L phases They prepared labeled antiglobulin against each phase separately and exposed each phase to each of the globulins Similar peripheral staining of all stages occurred in all cases Sorption experiments however indicated that the three phases were not identical serologically

Levina (1958) showed by precipitin reactions that unlabeled antisera and labeled antiglobulin to *Bacillus anthracis* were specific for *B anthracis* in comparison with anthracoides organisms Labeled globulin stained the homologous species but not others although the precise identity of the anthracoides gram positive and intestinal bacteria tested was not given

A more detailed study of *B anthracis* was carried out by Cherry and Freeman (1959) who prepared labeled antiglobulin to encapsulated cells of this species This reagent stained capsules of mucoid cells in a brilliant and distinctive pattern Nonencapsulated cells stained less brilliantly with cell walls and septa showing greatest fluorescence Intracellular spores did not stain but free spores showed fluorescence of a lower level than either encapsulated or nonencapsulated vegetative cells *Bacillus cereus* and encapsulated *Bacillus megatherium* vegetative cells stained to varying degrees with anti-*anthracis* globulin and it was not possible to

eliminate this cross reaction completely by any sorption procedures tried *B anthracis* in tissues of experimentally infected animals and in three human cases of inhalation anthrax were readily demonstrated with labeled globulin Formalin fixed and paraffin embedded sections could be stained as well as impression smears Organisms showed brilliant fluorescence in sections when stained as long as one year after the sections were pre pared Staining with fluorescent antibody was considerably more sensitive in demonstrating organisms in tissues than the Brown and Brenn procedure for bacteria or routine hematoxylin eosin staining

Winter and Moody (1959a) prepared separate antisera against *Pasteurella pestis* whole cell and somatic substance antigens Agglutination tests with 29 strains of the homologous species and 19 strains of *P pseudotuberculosis* demonstrated a high degree of specificity for the antisera Fluorescein labeled globulin fractions of the sera gave brilliant specific staining of *P pestis* in dried smears from cultures or from infected animals (Winter and Moody 1959b Moody and Winter 1959) The pattern of results indicated that it was envelope antigen which stained with whole cell antibody Labeled antisomatic globulin stained cells if envelope substance was not present because of cultural conditions or because of treatment with heat or ethanol Neither *P pseudotuberculosis* nor 48 strains of other bacterial species were stained by anti whole cell globulin When mice were infected with as few as 250 plague bacilli it was possible to demonstrate organisms 2 days later by staining impression smears of viscera Cultural and serologic confirmation of staining results were not obtainable for another 3 days This finding has significant implications for practical use of the staining procedure in rapid diagnosis of *P pestis* in man or in field trapped animals

A comparison of the minimum number of cells needed for accurate identification by fluorescent antibody staining and by agglutination techniques was made by Moody *et al* (1959) They studied pure cultures of *Brucella* spp *Bacterium tularense* *Pasteurella pestis* *Malleomyces pseudomallei* *Vibrio comma* and *Streptococcus pyogenes* group A using homologous labeled and unlabeled antisera for staining and agglutination tests respectively In addition *Serratia marcescens* was added to bacterial suspensions to investigate effects of a contaminant Generally agglutination tests required at least 10^5 homologous cells per milliliter for a positive reaction in contrast to staining which required only 40 to 100 organisms per milliliter Presence of the contaminant tended to obscure agglutination reactions whereas fluorescent staining was unaffected

Other bacterial species the specific staining of which has been reported briefly are *Shigella flexneri* (La Brec *et al* 1958) *Pasteurella tularensis*

(White and Blundell 1958) *Listeria monocytogenes* (Smith *et al* 1959) and *Erysipelthrix insidiosa* (Marshall *et al* 1959)

Vogel and Padula (1958) used the indirect staining procedure to demonstrate antibodies in patients infected with *Histoplasma capsulatum* *Candida albicans* *Blastomyces dermatitidis* and *Cryptococcus neoformans* All the fungi exhibited slight bluish green autofluorescence but upon being stained specifically each species showed a characteristic fluorescent picture When unlabeled normal serum was employed as primary reagent instead of unlabeled antiserum some degree of staining resulted with all antigens In one case of cryptococcal meningitis the patient s serum was negative for antibodies by several other serologic procedures but was positive in the staining reaction Because of the small number of cases and strains involved in this study it is not possible to draw conclusions about general applicability of the methods employed for detecting antibodies in fungal infections

In the first and at present only systematic survey of staining reactions of fungi with labeled antibody Gordon (1958) obtained the following results 28 strains of *Candida albicans* and 8 strains of *Candida tropicalis* yielded strong fluorescence with anti *C albicans* globulin 67 strains belonging to 6 other species of *Candida* and 15 other genera of yeastlike organisms stained weakly or not at all Anti *Histoplasma capsulatum* stained the homologous species strongly but not *Blastomyces dermatitidis* or *B brasiliensis* Anti *B dermatitidis* stained yeast phases of the homologous fungus and of *C albicans* strongly but did not stain *B brasiliensis* or *H capsulatum*

Encouraged by the high degree of specificity exhibited by labeled antiserum to *Candida albicans* Gordon (1959) investigated in detail the reactions of anti *Histoplasma capsulatum* serum He found that labeled globulin derived from rabbits infected with the latter species was capable of differentiating this species from tissue forms of other pathogens including *Blastomyces dermatitidis* *Cryptococcus neoformans* *Sporotrichum schenckii* *Leishmania donovani* and *Toxoplasma gondii*

Moulton and Howarth (1957) demonstrated *Leptospira canicola* in kidney sections of infected hamsters by specific staining with labeled homologous antiserum Distribution of organisms was similar to that shown by Levaditi s silver stain on alternate sections They also stained impression smears prepared from membrane filters through which cultures of leptospira had been filtered

Detection of antibodies to *Treponema pallidum* by the indirect staining procedure was investigated by Deacon *et al* (1957) Antigen consisted of smears of dried spirochetes from testicular tissue of infected rabbits

Serial bleedings from three experimentally infected rabbits and human sera from laboratory stocks were tested by this method in comparison to three other serologic procedures for syphilis detection. A varying pattern of positive results was obtained but the number of sera reported was too small to allow general conclusions to be drawn as to broad applicability of the test in syphilis serology.

C. PROTOZOA AND HELMINTHS

Differentiation of two morphologically similar species of parasitic amebas *Entamoeba histolytica* and *E. coli* by staining with specific labeled anti globulin was investigated by Goldman (1953, 1954). Each species of cultured ameba stained most brightly with homologous antibody but a lesser degree of cross staining also occurred with heterologous antiglobulin. When various combinations of the two species (alone, together, or mixed with other amebas) were exposed to labeled antibody it was possible to describe the contents of each mixture on the basis of staining results in terms of the presence or absence of one or both species. Staining tests with four other species: small race *E. histolytica* (= *Entamoeba hartmanni*), *Entamoeba invadens*, *Dientamoeba fragilis* and *Endolimax nana* resulted in minimal fluorescence with both anti *E. histolytica* and anti *E. coli* conjugates. Conventional methods for estimating brightness visually were employed in these experiments. To facilitate more refined studies of antigenic relationships in this group of parasites a micro fluorimeter was employed to measure the brightness of individual amebas stained with labeled antibody (Goldman, 1960). By standardized pro cedures for preparing, staining and measuring organisms it was shown that *E. hartmanni*, considered by some to be only a size variant of *E. histolytica*, was as different antigenically from four strains of *E. histolytica* as were *E. coli* and *Entamoeba moshkovskii* (Goldman, 1959). It was also shown that a possible antigenic difference existed between three cultured strains of originally invasive *E. histolytica* and one originally noninvasive strain of the same species.

Toxoplasma gondii has been stained specifically in smears of peritoneal exudate from acutely infected mice (Goldman, 1957a) and in frozen or paraffin embedded sections of mouse tissues (Carver and Goldman, 1959). In spite of the small size of individual parasites they were readily demon strated in sections of spleen, liver, lung and brain. In the case of mice infected with a chronic strain of *Toxoplasma* cyst stages were stained in brain tissue. Specific fluorescence of the cyst wall supported the hypothesis of parasite origin of this structure (Lainson, 1958).

Smears of formalin washed toxoplasms have been used as antigen in a

serologic test for antibodies to this species using the inhibition staining procedure (Goldman 1957b) A comparison of results obtained by this test and by the methylene blue dye test (Sabin and Feldman 1948) on 230 sera showed good parallelism in identifying positive and negative reactors with the fluorescence test somewhat less sensitive than the dye test However the fluorescence test was simpler to perform and used a dead relatively stable antigen in comparison to the live unstable antigen used in the dye test

Because of difficulties associated with identification of *Anaplasma marginale* in erythrocytes of cattle with low grade infections Ristic *et al* (1957) prepared labeled anti *Anaplasma* globulin for use as a specific stain for the organism With this reagent it was possible to demonstrate *Anaplasma* in alcohol fixed blood smears A carrier animal whose blood was negative by Giemsa stain showed several *Anaplasma* like bodies when stained with specific antiglobulin Fluorescent staining was not evaluated in comparison with conventional methods on groups of known and unknown infections

Beale and Kacser (1957) attempted to localize antigenic sites of *Paramecium aurelia* by exposing intact living or killed paramecia to labeled specific antiserum Distinctive reactions occurred with homologous but not with heterologous serotypes even though the latter were in the process of conjugation with homologous individuals It was concluded from the distribution of specific fluorescence that immobilization antigen was not of ciliary origin but was rather an exuded fluid substance covering the whole surface of the paramecia

McEntegart *et al* (1958) were able to demonstrate differences in staining reactions between *Trichomonas vaginalis* and *T foetus* using specific antiglobulin to each species When antiglobulin to one strain of the former species was used to stain a different isolation of the same species reduced fluorescence was observed The extent to which this demonstrated real strain distinction could not be determined on the basis of these preliminary observations

Fife and Muschel (1959) used the indirect staining procedure as a serologic test for antibodies to *Trypanosoma cruzi* Antigen consisted of washed formalin killed *T cruzi* obtained from cultures Since hemoflagellates dried on slides stained nonspecifically with normal serum testing was done with suspensions of antigen in which condition nonspecific staining was not a problem A comparison of fluorescent antibody and complement fixation tests on 130 human sera showed the former test to be slightly more sensitive in detecting infections However the fluorescence

procedure also showed some weak reactions in noninfected individuals who were negative by the complement fixation reaction

Jackson (1959) exposed living intact adults and larvae of *Trichinella spiralis* to fluorescein labeled antibodies derived from rabbits undergoing infections with this helminth In addition sections of adults and larvae occurring in rats and mice were studied The external precipitates which ordinarily form around the oral anal and vulval orifices of living worms incubated in specific antiserum were highly fluorescent when labeled antiserum was employed Neither internal tissues nor cuticle was stained while the worms were alive The digestive tract of sectioned worms was stained by all of the labeled antisera but reproductive organs and musculature were stained by some sera and not by others In no case did the cuticle stain These results were taken to indicate that excretions and secretions from the digestive tract were the most common antigenic stimulants in infected rabbits Antibody responses to antigens from other worm tissues developed less regularly depending perhaps on the number of parasites undergoing dissolution in the host

IV Summary

The fluorescent antibody literature which is concerned with viruses and bacteria is sufficiently extensive and varied to indicate the types of investigation with these agents which are likely to be profitable in the field of tropical medicine However it may be appropriate here to comment briefly on the possible role of labeled antibodies in the fields of protozoology and helminthology

Precise identification of serotypes in bacteria and in protozoa such as *Paramecium* has thrown much light on previously obscure epidemiological and biological patterns It is reasonable to assume that similar knowledge about parasitic amebas hemoflagellates and *Plasmodium* would have a similar enlightening effect Serologic studies with fluorescent antibody in *Salmonella* (Thomason *et al* 1957) *Escherichia coli* (Whitaker *et al* 1957) *Rickettsia* (Goldwasser and Shepard 1959) *Paramecium* (Beale and Kaeser 1957) and *Entamoeba* (Goldman 1959) indicate that this new methodology is especially well suited for such investigations Few organisms are needed to serve as antigen and the presence of gross contamination with other organisms environmental debris or host tissue may not have any significant effect on reactions with the organism being studied

The size and morphological complexity of most helminth parasites make it unnecessary to resort to fluorescent antibody for identification purposes However larval filariae in blood or in the mosquito host and

obscure lesions such as occur in eosinophilic lung may be more readily identified with the aid of specific labeled antibody than by other means In addition the fine serology of large parasites e g antigenicities of different structures in *Ascaris* are likely to be more readily and precisely studied by microscopic immunochemistry than by grosser methods

Serology and microscopy are fundamental tools for the biologist Application of the combined tool labeled antibodies to problems in tropical medicine can serve only to increase greatly scientific knowledge in this area

Appendix

1 *Illuminators for Fluorescence Microscopy*

American Optical Company Buffalo 15 New York
Ernst Leitz G.m.b.H Wetzlar Germany
C Reichert A G Hernalser Hauptstrasse 219 Vienna XVII Austria
H Wild Surveying Instruments Supply Co Ltd Heerbrugg Switzerland
Carl Zeiss Oberkochen Germany

Labeling Reagents

a Fluorescein amine fluorescein isothiocyanate rhodamine B isothiocyanate and labeled antisera are available from the following dealer Since catalogue items change periodically not all reagents are equally available from all distributors

Baltimore Biological Laboratory 2201 Ai qu th Street Baltimore 18 Maryland
Dajac Laboratories 5000 Langdon Street Philadelphia 24 Pennsylvania
Delta Chemical Works 23 W 60th Street New York 23 New York
Nutritional Biochemicals Corp 21010 Miles Ave Cleveland 28 Ohio
Roboz Surgical Instrument Co 4500 Wisconsin Ave NW Washington D C
Sylvana Chemical Company Orange New Jersey

b 1 Dimethylamino naphthalene 5 sulfonyl chloride may be obtained from the California Foundation for Biochemical Research Los Angeles California

c Nuclear fast red may be obtained from George T Gurr Ltd London S W 6 England

d Lissamine rhodamine B 200 is available from Imperial Chemical Industries Dyestuffs Division Manchester England

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Ticks and Tick Borne Diseases Some International Problems and Cooperation in Their Study[1]

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Few phases of medicobiological science impinge upon so many interdisciplinary and international boundaries as research on ticks and tick borne diseases of man and animals In many parts of the world but especially in the vast areas of developing countries problems of expanding and improving animal resources are correlated with those of improving human nutrition providing resistance against disease and increasing living standards The most important tick parasites of livestock usually also vectors of human diseases interest public health and veterinary specialists alike Workers in the fields of human and veterinary medicine frequently so closely collaborate and cooperate in studies on zoonoses that their colleagues are sometimes troubled to recall whether they are human doctors or animal doctors When wild bird or mammals serve as hosts of one or more developmental stage of ticks field laboratory and museum biologists play a significant research role Virologists rickettsiologists bacteriologists and protozoologists become involved owing to ticks unique ability to harbor and transmit a considerable variety and number of pathogenic organisms

The tremendous amount and range of tick infested and disease infected animal movement occurring daily in many areas of the world is seldom realized Herds travel great distances often across provincial or national boundaries in search of water and grazing Animals are transported on fast railroads and ships to populous urban centers for slaughter and by air for exhibition or stock improvement Pack animals trek across deserts and mountain ranges Wild animal migrations are in some instances equally numerous and far reaching Millions of migrating birds rapidly flying between continents may be virus infected and tick infested Practically no economically important tick species is confined to a single nation no matter how large it may be Through one or more agencies populations of the world s most serious tick species have become established far from their original endemic area

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[1] The opinions and assertions contained herein are the private ones of the author and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large From Research Project NM 52 08 03 3 Bureau of Medicine and Surgery Navy Department Washington 25 D C

One of the most fascinating examples of this scientific and geographical gamut is the tick vectored Russian Spring Summer (RSS) complex of Asiatic European viruses The *antigenic characteristics* of *these* viruses are identical or quite similar but their disease symptoms and epidemiological patterns differ greatly The classic northern Asiatic forest RSS encephalitis a paralytic polioencephalomyelitis has geographical forms of varying severity and mortality (Smorodintsev 1958) A biphasic meningoencephalitic form with a low mortality rate occurs in forest areas of European U S S R and parts of Austria (van Tongeren 1955 van Tongeren *et al* 1955 Verlinde *et al* 1955 Pattyn and Wyler 1955) A third syndrome louping ill an epizootic encephalomyelitis of sheep is found in pastures in Scotland and northern England where unlike the other forms man is seldom involved (Smorodintsev 1958) Another subgroup of the RSS complex causes various types of acute infectious hemorrhagic fevers with differing pathogenicity tissue tropism and epidemiology in Crimea Siberia and Uzbekestan Central Asia (Bilibin 1950 Gajdusek 1953 MacLeod *et al* 1956 Konowalchuk *et al* 1957)

All RSS complex viruses are tick borne *Ixodes ricinus* (Linnaeus) *I persulcatus* Schulze *Haemaphysalis concinna* Koch *Dermacentor silvarum* Olenev *D pictus* Hermann and *Hyalomma marginatum* Koch [= *H plumbeum* (Panzer)] have been incriminated as tick hosts and vectors of RSS complex viruses *Hyalomma detritum* Schulze and *Rhipicephalus turanicus* Pomerantzev have also been suggested as vectors The European biphasic disease is also acquired by drinking virus infected milk of goats bitten by infected ticks Every tick species differs in host predilection in each of its three developmental stages as well as in seasonal activity temperature and humidity requirements geographic distribution and many other factors Vertebrate hosts parasitized are many kinds of small rodents various squirrels hares elks wolves all domestic mammals man and a large variety of birds Ecological zones infected are Siberian cedar and broadleaf forests European pine and broadleaf forests and Crimean and Siberian grassy lake and wooded steppe areas

For years these diseases were believed to be confined to specific areas of the U S S R nearby eastern and Central Europe and restricted localities of the British Isles In 1954 however disturbing reports of neutralizing antibodies to RSS virus in 6 of 26 human sera from Saurashtra peninsula Bombay State India suggested human RSS virus infection far south of its previously known geographical and ecological range (Smithburn *et al* 1954 Kerr and Gatne 1954) In Malaya Smith (1956) provided another surprise by isolating a RSS group virus (TP21) from tropical forest insectivore and rodent ticks *Ixodes granulatus* Supino

The same year an epidemic of a prolonged prostrating febrile and often fatal human illness Kyasanur Forest disease (KFD) broke out in a Mysore India village adjacent to a tropical forest in which monkeys were also dying (Work and Trapido 1957 Work *et al* 1957) Fortunately a virus research laboratory sponsored by the Indian Council of Medical Research and the Rockefeller Foundation was then being established at Poona Rapid deployment of highly qualified versatile Indian and American scientists experienced in clinical medicine virology entomology ornithology mammalogy and parasitology provided within a few months a unique and remarkable epidemiological account of this and subsequent KFD epidemics in Mysore forest edge villages (Work 1958)

In India where descriptions of disease symptoms and epidemics are part of tradition these explosive KFD outbreaks were entirely new and different from any illness in contemporary written or legendary history Death of two species of wild monkeys in forests suggested sylvan yellow fever far from its known American and African range A week or so after having visited these forests usually to gather wood villagers suddenly succumbed to a severe febrile illness Among a variety of symptoms extensive hemorrhea was prominent This syndrome suggestive of Omsk hemorrhagic fever was soon proved by the Poona Laboratory to be caused by a RSS virus closely related to the Siberian type Besides monkeys palm squirrels and forest shrews possessed neutralizing antibodies Endemic ticks *Haemaphysalis spinigera* Neumann common on forest monkeys and birds and also parasitizing the palm squirrel were a source of KFD virus

Work (1958) concluded his classic review of two seasons intensive study of KFD epidemiology by saying preliminary clues indicate a possible mechanism for introduction of the virus into this newly infected area from elsewhere in India if not from north of the Himalayas Certainly enough evidence exists by commonplace finding of *Haemaphysalis spinigera* on birds in Kyasanur Forest and repeated isolations of KFD virus from this tick species to suggest that tick infested birds may play an important role in the spread of the virus to neighboring susceptible forest areas in India

Another outstanding intercontinental epidemiological subject concerns a complex of febrile exanthematous syndromes in most of Africa southern and southeastern Europe parts of the Near East and India Related to Rocky Mountain spotted fever of the Americas (Badger 1933 Hass and Pinkerton 1936) a single agent *Rickettsia (Dermacentroxenus) conorii* Brumpt 1932 is tentatively believed by Steinhaus (1947) and others to

cause the diseases referred to as *fièvre boutonneuse* Marseille fever *fievre eschonodulaire* eruptive fever South African tick bite fever tick typhus Pretoria ten day fever East African typhus Kenya typhus Abyssinian typhus *etc* Indian tick typhus is also caused by *R conorii* (Philip *et al* 1950 Philip 1952) Although fatalities are few patients suffer severe pain and debility South African workers maintain that the local strain differs sufficiently to warrant subspecific status variety *pijperi* (Do Amaral and Monteiro 1932 Mason and Alexander 1939 Pijper and Crocker 1938 Gear 1954)

Bits and pieces of experimental and circumstantial evidence in scattered areas of the three affected continents incriminate numerous vertebrate reservoirs and arthropod vectors of this zoonosis In southern and southeastern Europe and northwestern Africa domestic dogs and kennel ticks *Rhipicephalus s sanguineus* (Latreille 1806) appear generally to be involved However in southwestern France and Italy Sigalas and Lamontellerie (1954) and Berri (1953) respectively suggest that the evidence incriminating ticks is so slight that other insects should be examined for their ability to vector the rickettsia Populations of *R s sanguineus* in northwestern Africa are considered to be more aggressive toward man than those elsewhere in Africa Europe or Asia but the direct evidence is flimsy Research adequate to determine the role of noncanine vertebrates as reservoirs has not been undertaken in Europe or northwestern Africa although rickettsiae have been recovered from a few dogs in these areas In Cairo and its environs where dogs and kennel ticks are extremely common no cases of boutonneuse fever have been identified

The work of Heisch *et al* (1957) in the Nairobi area of Kenya casts much doubt on the role of dogs as domestic reservoirs of *Rickettsia conorii* and incriminates in their place field rodents of the genera *Otomys* and *Lemniscomys* No infected kennel ticks *Rhipicephalus s sanguineus* were found but *Haemaphysalis leachii* (Audouin) and *Rhipicephalus s simus* Koch were infected and a single strain was isolated from *Amblyomma variegatum* (Fabricius) Inoculation of strains from ticks into human volunteers produced symptoms varying from mild to severe These results contradict those of Roberts (1935) who was convinced after isolating *Rickettsia conorii* from kennel ticks around Nairobi that *Rhipicephalus s sanguineus* is the chief if not the only vector here In Ethiopia however Reiss Gutfreund (1956) concludes from limited observations that *R s sanguineus* and *R simus simus* are the vectors

Boutonneuse fever enzootic in South Africa is intimately associated with the veld but occurs also in urban suburbs Local workers consider it

probable that most species of ixodid ticks are capable of transmitting the disease and specify especially *Amblyomma hebraeum* Koch *Rhipicephalus appendiculatus* Neumann *R e evertsi* Neumann *Haemaphysalis leachi* and *Hyalomma truncatum* Koch [= *aegyptium* (*sic*)] (Gear 1954) Here also field rodents *Rhabdomys* sp and *Otomys* sp as well as commensal rodents *Rattus rattus* were found to be infected Dogs may play a minor epidemiological role but their actual degree of importance does not appear to have been sufficiently investigated In the absence of vertebrate hosts the rickettsia can be transovarially transmitted indefinitely in *Haemaphysalis leachi* and probably in other species of ixodid ticks (Gear and de Meillon 1941 Gear 1954)

Indian tick typhus is thus far associated only with a few dogs and strains of their common tick parasites *Rhipicephalus s sanguineus* The epidemiological picture in the Congo is wildly confused A Siberian form appearing to belong to this etiological group and occurring in ground squirrels and other rodents is transmitted to man by *Dermacentor nuttalli* Olenev *D silvarum* Olenev and *Haemaphysalis concinna* Koch (Korshunova 1943 Bocharova 1943 Shkorbatov 1944 Krontovskaya and Shmatikov 1943) Infected larvae have been collected from Siberian rodents and infected adults were taken from cattle and dogs

Epidemiological facets in none of the above mentioned instances have been satisfactorily elucidated Comparative studies of various strains the importance of transovarial transmission of the etiologic agent in different tick hosts and under dissimilar ecological conditions the question of vectors other than ticks and the role of domestic dogs and/or commensal and wild animals as vertebrate reservoirs are some of the chief factors awaiting local study and evaluation

While the epidemiology of boutonneuse fever remains poorly known many aspects of another widely distributed acutely febrile rickettsial disease Q fever caused by *Rickettsia* (*Coxiella*) *burnetii* Derrick 1937 are well studied Recent reviews and textbooks stress inhalation of infected dust consumption of infected milk products and contact with infected animal parts as sources of the organism causing numerous dramatic outbreaks in military personnel schools institutions laundries and slaughterhouses Unrecognized before 1935 the agent of this world wide disease was in early stages of investigation frequently recovered from *Haemaphysalis Ixodes Dermacentor Amblyomma Boophilus* and *Hyalomma* ticks and transovarial transmission was demonstrated in several species Wild mammal and bird reservoirs were also discovered After the initial brief flurry of interest in this disease as an enzootic phenomenon the attention of practically all researchers public health authorities writers

and reviewers turned to the epizootic aspects of transmission The basic natural history of the disease tantalizingly suggested by early workers remains not only almost entirely unknown but also of little or no interest to contemporary researchers Yet in this ever shrinking world with its increasingly traveled population and fast vehicles exposure of nonimmune persons to the enzootic disease constantly widens to a degree incomprehensible a generation earlier Of considerable human health significance this easily studied phase of Q fever epidemiology should attract rickettsiologists veterinarians zoologists parasitologists and quarantine and public health authorities to reveal the role of wild animals and ticks as reservoirs in nature

Owing to the expense and numerous other problems confronting the still limited virological facilities of the world surprisingly little research into viruses in ticks has thus far been undertaken The value of attempts to isolate viruses from tick species in general and especially from those parasitic on birds was illustrated in Egypt by isolation of several viruses in two or more new groups from *Argas persicus* (Oken) and *A hermanni* Audouin tick parasites of birds (Taylor and Hurlbut 1958) One of these new tick viruses was also isolated from children with acute febrile illness Sera from 4 to 16% of village residents possessed neutralizing antibody suggesting past infection with this or a closely related virus An apparently important bird host of this virus in Egypt is the cattle egret or buff backed heron *Bubulcus i ibis* which has recently become established in the United States where *Argas persicus* also occurs Several still unstudied Indian tick viruses have been isolated as well (Work 1959)

West Nile (WN) virus first isolated from a human being in Uganda (Smithburn *et al* 1940) and demonstrated in indigenous populations of Uganda Sudan Kenya and Belgian Congo (Smithburn and Jacobs 1942 Smithburn 1952) is now known from epidemiological studies in Egypt and the Sudan to be a common bird mosquito man infection (Taylor *et al* 1956) WN virus has been isolated from naturally infected *Argas* ticks in Egypt and three tick species *Ornithodoros savignyi* (Audouin) *O erraticus* Lucas and *Argas persicus* (Oken) become infected during experimental feeding on infected animals *O savignyi* after parenteral infection transmitted the virus when biting infant mice and the virus was isolated from its coxal fluid (Hurlbut 1956) Extensive epidemics of WN fever have occurred among nonimmune immigrants in Palestine (Bernkopf *et al* 1953 Goldblum *et al* 1954) and the virus has recently been isolated from human beings in India (Work 1959) Apparently sporadic infections of WN virus occur in

the dry grassy central plateau of South Africa where owing to cold and dry winters endemic mosquito infections are unlikely (MacIntosh per sonal communication 1959) This suggests introduction by migrating birds of a bird mosquito adapted virus into new areas of susceptible human beings and a possible role of bird ticks in virus maintenance in nature

Numerous birds throughout the world have been found as tick hosts during migration and while resident in virus infected areas An endemic African tick *Hyalomma rufipes* Koch associated with many human and animal pathogens (Hoogstraal 1956a) was postulated by Pomerantzev (1950) to have been introduced and established in certain Russian areas through the agency of migrating birds At least twenty kinds of migrants from tropical Africa to eastern Europe and Russia carry *H rufipes* as they pass over Egypt (Hoogstraal and Kaiser 1958a Hoogstraal *et al* in press) Similarly scattered exotic populations of a European Asiatic tick species *Hyalomma marginatum* Koch incriminated in the epidemiol ogy of RSS virus and other pathogens have been found in Africa Birds were suspected but not known to carry this tick species into Africa until infested migrants were recently recovered in Egypt This source is also responsible for the periodic establishment of *H marginatum* populations far north of their usual range in U S S R (Kurchatov 1939)

Endemic polar and widely ranging oceanic birds are common hosts of *Argas Ornithodoros* and *Ixodes* ticks but their relation to viruses re mains unknown

Owing to the number and variety of opportunities for transport of ticks knowledge of species distribution is essential The scope of this in formation is rapidly increasing in many parts of the world Some out standing recent examples of faunal generic or geographic reports are Dumbleton (1953) for New Zealand Roberts (1953) for *Aponomma* and *Amblyomma* of Australia Kohls (1950) for the Philippines Kohls (1957a) for Borneo and Malaya Toumanoff (1944) for Indochina Anas tos (1950) for Indonesia Kohls (1953) for Guam Kohls (1957b) for Micronesia Keegan and Toshioka (1957) for Japan Korea and Ryukyu Islands Pomerantzev (1950) and Serdyukova (1956) for U S S R Po pelova Shtrom (1953) for argasids of U S S R Galuzo *et al* (1958) for Kazakhstan and Central Asian republics Hoogstraal and Kaiser (1958c) for Iraq Hoogstraal and Kaiser (1959) for Arabia Kurtpinar (1954) for Turkey Pandazis (1947) for Greece Enigk (1947) for south eastern Europe Starkoff (1958) for Italy Hoogstraal and Kaiser (1958b) for Egypt Morel (1958) for French West Africa (domestic animals) Tendeiro (1952) for Portuguese Guinea Pierquin and Nieme

geers (1957) for Belgian Congo and Ruanda Urundi Hoogstraal (1956a) for Sudan Theiler and Robinson (1954) for Angola and Rhodesia Zumpt (1958) for Bechuanaland Theiler (1941 1943 1945 1947 1948 1949a b 1950a–c 1956) and Theiler and Robinson (1953a b) for Union of South Africa (certain species) Hoogstraal (1953) for Madagascan archipelago Gregson (1956) for Canada Cooley and Kohls (1944) for argasids of North and Central America and Cuba Cooley (1938) for *Dermacentor* and *Otocentor* of the United States Cooley and Kohls (1945) for *Ixodes* in North America Bishopp and Trembly (1945) for North America (certain species) and Aragão (1936) for Brazil

These faunal geographic or generic studies in many instances provide valuable background data on ticks in a specific area The data for a few species however are sufficiently numerous accurate or precise to provide information adequate in scope for planning and executing scientific *and economic tick control programs or epidemiological studies For a* much studied superfamily (Ixodoidea) in which no more than 700 species are now known to exist a surprising number of perplexing and complex systematic problems remain Biological ecological and physiological studies commensurate with their considerable economic importance have been accomplished for *very few of the world s common tick species*

Each species deserves serious study Species are important because they represent an important level of integration in living nature This recognition is fundamental to pure biology no less than to all subdivisions of applied biology Whether he realizes it [or not] every biologist—even he who works on the molecular level—works with species or parts of *species and his findings may be influenced decisively by the choice of a* particular species The communication of his results will depend on the correct identification of the species involved and thus on its taxonomy (Mayr 1957)

A case in point and a further example of both the interdependence of taxonomic biological and epidemiological studies and of the need for international and interprofessional cooperation is the present systematic status of the well known African relapsing fever vector *Ornithodoros moubata* (see Hoogstraal 1956a pp 119–190) Taxonomically *O moubata* was for over fifty years considered to be a settled matter Biologically a few perspicacious persons were uneasy about the relationships *between these ticks* chicken and man and between populations from human habitations and wart hog burrows or wild tortoises Epidemiologically it was noted with no further evidence of curiosity that some *O moubata* populations were consistently uninfected with spirochetes and existed *in the absence of relapsing fever* Economically a few public

health authorities cautiously questioned the necessity of spending large sums of public or industrial funds to eliminate all *O moubata* foci in their area

Walton s (1950 1953 1955 1957 1958a b 1959) work shows that the *O moubata* complex consists of three distinct groups in which morphological as well as physiological and biological differences will probably justify subspecific status The first domestic group including several minor biological variants inhabits human dwellings in East and West Central Africa The second wild group exists in wart hog burrows from the Sudan to the Transvaal The third group occurs in human dwellings in the Union of South Afri a and Angola The wild group is also found in some East African human dwellings even at high altitudes in smaller mountain ranges Early attempts to isolate and identify East African domestic variants was much impeded by this confusing factor

Three domestic variants were recognized A mammalian feeder was confined to cool damp highlands An avian feeder was widely distributed in warm and damp areas and a mixed feeder centered in arid areas

Behavior differences in *O moubata* populations markedly affected spirochete transmission On the Kenya coast a domestic strain fed almost exclusively on fowls in the absence of spirochetes In high rainfall areas of northeastern Kenya and northwestern Tanganyika highlands a strain with a high selectivity for human blood and existing in floors of huts transmitted spirochetes

Further south in the Usambara mountains and on the Tanganyika plateau tick infestation increased but relapsing fever decreased in huts containing fowls This relationship changed however in the central arid areas where tick density remained unaltered at all relative humidity (R H) values in the presence of fowls However in the absence of fowls density increased in proportion to decreasing R H values and relapsing fever again became endemic

Subsequent work revealed that pure lines of these biologically distinct populations could be obtained Individual characteristics of water loss survival rate under starvation number of nymphal molts host predilection and coxal fluid excretion were determined Morphological criteria such as coarseness of integumental mammillation number of setae length of tarsus form of Haller s organ and size of chelicerae were established and subgroups with distinctive hair lengths and egg size and color were correlated

This brief review of some results of painstaking research often at great risk in the midst of insurrection illustrates what a single worker can accomplish in a few years time Comparative collections of living material

furnished by colleagues in other areas supplemented what could be collected personally Preliminary data of outstanding significance to epidemiologists public health officials biologists and taxonomists have been made available but much additional information remains to be obtained Similar problems of mutual professional and regional interest have been met in other fields here is a comparable challenge awaiting acceptance

Although *Ornithodoros moubata* is an example of a single vector species whose long settled systematic status has suddenly become moot the taxonomic position of practically all species in certain entire ixodid genera remained questionable until very recently when reliable specific criteria were at last developed The genus *Hyalomma* as an example and the interdependence of taxonomic and epidemiologic research was discussed at the Sixth International Congresses on Tropical Medicine and Malaria (Lisbon 1958) (Hoogstraal and Kaiser in press)

Hyalomma ticks ranging from the Atlantic shores of southern Europe and of Africa through the Near and Middle East to India are pre eminent reservoirs and vectors of a wide variety of pathogens causing human and animal diseases (e g Q fever tularemia at least two kinds of hemorrhagic fevers Spring Summer and other mosquito borne encephalitides boutonneuse fever plague brucellosis theileriasis of cattle and camels equine and bird piroplasmosis and theileriasis rickettsiae pathogenic for guinea pigs and sporozoa of tortoises) Injuries resulting from *Hyalomma* bites are tick paralysis anemia sloughing of host skin causing abcesses liable to secondary infection sweating sickness of cattle footrot and lameness and paralysis of sheep and calves As research continues other diseases and injuries will undoubtedly be added to this list

In spite of their economic importance little effort has been made fully to define *Hyalomma* species and their range and limits of variation Evaluation and practical application of results of scores of incidental and monumental taxonomic biological and epidemiological studies referring to a jumble of unrecognizable *Hyalomma* appellations are largely worthless owing to uncertainty of species with which they deal Applied researchers have wasted considerable time money and resources by overlooking the necessity of being able to identify describe and differentiate *Hyalomma* species

In Koch s (1844) *Hyalomma* review sixteen species were recognized Because of their great superficial variability Neumann (1911) reduced these to four species Between 1919 and 1950 the Schulze school named some eighty forms mostly by means of a few ambiguous sentences Delpy (1946) commenced to rear progeny from single females and made notable preliminary contributions toward stabilizing *Hyalomma* taxonomy When

the problem became too vast for his facilities he synonymized literature descriptions and illustrations under a dozen species (1949a b) During the same period Adler determined the highly specific diagnostic value of the female genital area (Adler and Feldman Muhsam 1946 1948) Hoogstraal (1956a) showed the practical application of this character in identification of field collections

Discovery of the diagnostic value of the structure of the *Hyalomma* female genital area has proved to be an immense boon to tick taxonomy Even in this genus however other morphological and biological characteristics require evaluation before individual species can finally be established Females of certain species widely separated geographically and with greatly differing host preferences seasonal periodicity and other features are practically indistinguishable on the basis of this character alone When applied to other taxonomic groups the over all range of specific and group genital area variation must be even more carefully studied in the initial phases Such investigation is a slow one requiring numerous samples from widely scattered geographical areas and laboratory rearing of progeny from representative females from a number of areas Here museum type studies of any preserved specimens that happen to be available are largely worthless Experience shows that there is no substitute for extensive personal collection and observation by the individual researcher combined with begging and interesting persons in the field in other regions to make every effort to obtain additional comparative series and data A conclusive study therefore results only after expenditure of time and energy by workers in several disciplines and countries

One fundamental approach to improving knowledge of tick taxonomy distribution seasonal activity host relationships and ecology is to collect data and specimens from all environments and hosts at each season of the year in the largest possible geographical area When results from these activities can be correlated with host surveys (Hoogstraal 1956b) they become additionally useful

Recently the Food and Agriculture Organization of the United Nations (FAO) asked its specialists in northern Africa and the Near East to do what they could in surveying their local tick fauna a program in which the U S Naval Medical Research Unit at Cairo cooperates by identifying specimens and offering suggestions and equipment Even the somewhat limited response that resulted from this request has provided a large amount of new and important biological and taxonomic data all of which are rapidly made known to colleagues everywhere

Many organizations such as ministries of public health and animal resources educational and research institutions game departments inter

national cooperation groups natural history museums collectors of entomological and vertebrate specimens and others have facilities within their existing framework to collaborate in surveys Centers where studies are already in progress are usually happy to accept trainees for this purpose In Africa and Asia where most of the world s poorly studied tick species and tick borne diseases of man and animals occur a number of research centers now devote much effort to solving outstanding problems on a regional rather than on a local basis

In southern Africa Dr G Theiler of the Onderstepoort laboratory and in eastern Africa Dr J Walker of the East African Veterinary Research Organization function as authorities for regional identification of ticks sources of suggestions for methods and techniques and centers for dissemination of knowledge The tremendous Indian subcontinent is being actively surveyed by Drs H Trapido and R Varma of the Virus Research Centre at Poona Numerous facilities for this purpose exist in the U S S R and Japan NAMRU 2 on Formosa is producing valuable information on the ticks of that and nearby islands

For the Americas the functions of the Rock Mountain Laboratory as a research reference and training center for tick borne disease problems have been a classic example of interprofessional and international cooperation for two generations

By collaboration within the limits of their available funds and existing resources organizations such as these have been directly or indirectly responsible for a large measure of knowledge of ticks and the diseases with which they are associated However they are also in the anomalous position of often having indicated the existence of more unrecognized problems than they can ever hope to solve The growing awareness of the public health importance of approximately a hundred different zoonoses and the international problems related to the appearance of new diseases require an enlargement and elaboration of existing collaboration and research facilities

How did Kyasanur Forest disease become so suddenly established in Mysore? Do migrating birds with circulating viruses and/or dropping infected ticks disseminate these viruses? What is the medical significance of TP21 virus of Malayan rodents? Do latent foci of KSS virus exist in other parts of the world? If they do what factors allow them unexpectedly to fulminate into severe epidemics among nonimmune human populations? And since similar subjects concerning other arthropod borne viruses associated with migrating birds and mosquitoes (rather than ticks) exist in the Americas Africa Australia and Asia what is the most practical method

of studying the wide dissemination and exotic establishment of arthropod borne viruses causing such debilitating and often fatal diseases?

When problems such as these with their far reaching geographical implications and complex scientific interrelations become apparent we are fortunate in having the unique facilities of the United Nations and its organizations devoted to world health and agriculture to stimulate correlate and advise workers institutions and governments everywhere

Promptly meeting the international and interdisciplinary challenge posed by Kyasanur Forest disease and its related viruses the Sections of Endemo epidemic Diseases and of Veterinary Public Health of the World Health Organization (WHO) called to Geneva in March 1959 an advisory panel of specialists in clinical medicine virology ornithology bird migration and medical zoology from Britain Egypt Germany India South Africa U S A and U S S R Members were asked to consider the advisability of a world wide coordinated research program devoted to birds as disseminators of arthropod borne viruses after determining (1) information already available (2) degree of cooperation available throughout the world (3) what might be accomplished through established facilities (4) how international research on this subject can best be stimulated and implemented and (5) how new knowledge might best be disseminated

The advisory group was sufficiently impressed with the amount of circumstantial evidence to suggest that the unique international and interprofessional facilities and resources of WHO should be utilized to sponsor and coordinate an international research program devoted to exploring the actual role of birds as disseminators of arthropod borne viruses To do this requires supplementation of present ornithological facilities and establishment of certain new ones to study pertinent biological problems and provide sources of blood and tissue for virological examination Ornithological assistance need be furnished to established virological institutions Also suggested was short term or long term training for technical and professional aspects and eventually meetings of persons implementing and interested in the project in specific geographical areas to exchange information arrange interdisciplinary and international cooperation and plan the future research course An example of useful administrative assistance was the sponsorship of preparation and publication of a comprehensive detailed book reviewing biology ecology taxonomy and known disease relationships of all mosquitoes species known to bite birds The extensive relationships between mosquitoes ticks and resident or migrating virus infected birds require considerable field and laboratory study Also important would be the sponsoring of standard mapping of geographic distribution of each type of pertinent virus each species or

subspecies of relevant bird and each species of tick or mosquito that may be epidemiologically significant together with standard climatic vegetation and *demographic maps on which species maps can be superimposed* when required

In the introductory paragraph recognition was given to the need for *increasing and improving* animal resources especially in developing nations of the world in order to control zoonoses provide much needed revenue supplement protein poor human diets provide resistance against disease and increase living standards of indigenous populations Many researchers scientific institutions governmental agencies and intergovernmental organizations now appreciate this approach The first Institute on Veterinary Public Health Practice was held during October 1958 at University of Michigan s School of Public Health The World Health Organization has established a section of Veterinary Public Health in which study of tick borne disease control is an integral part At the London 1958 meeting of the Expert Panel on Tick borne Diseases of Livestock convened jointly by the Food and Agriculture Organization of the United Nations (FAO) and the International Office of Epizootics (OIE) a medical biologist was asked to represent WHO Later at the WHO conference *on birds as virus disseminators briefly described above he represented* FAO

At the joint FAO/OIE conference specialists in veterinary medicine *virology parasitology zoology and medical zoology from Australia* Belgian Congo Belgium Britain Germany France Iran Kenya Malaya Portugal Union of South Africa and United States served as members or observers to (1) review new knowledge in the field of tick borne diseases and their control (2) make recommendations concerning control of these vectors and diseases (3) consider deficiencies in knowledge of these subjects and (4) consider standardization of different aspects of investigations pertinent to these problems At this meeting the interrelations of medical and veterinary problems were frequently stressed

Another step forward in the international veterinary public health approach to solving disease questions was the establishment in 1956 of the Pan American Zoonoses Center in Azul Buenos Aires Argentina Supported by the Argentine government the Pan American Sanitary Bureau of WHO and the United Nations Technical Assistance Programme located in an important livestock region and assisted by the medical and veterinary professions livestock owners hospitals municipal authorities and other local groups this center includes laboratories offices classrooms library and farm annex Staff members consist of physicians veterinarians and members of allied professions In addition to research on major

zoonoses the center is a clearing house for information on all zoonoses and a training and educational institution for long term and short term fellowships special courses seminars and workshops Blood (1957) in concluding an announcement of the establishment of this activity states The needs and opportunities for research on the zoonoses are vast and the Center itself can be expected to cover only a very small part of this work Public and private institutions in all countries must therefore continue and intensify their own zoonoses research work which the Center [will] stimulate and coordinate

The considerable advancement toward the goal of improving human health and wealth stimulated by several international agencies even in these troubled times is a measure of progress of civilization and science International and interprofessional cooperation and free exchange of data and information lead to improved health greater economic and political stability and mutual human appreciation and understanding

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J

has caused it? The conception of the evil eye and the intervention of evil spirits or of the angry spirits of ancestors to whom proper respect has not been paid is still the overriding belief of a large section of mankind and is particularly a feature of tropical peoples These beliefs have not yet received the attention they deserve

Tropical diseases may be studied and described in the usual sections epidemiology transmission etiology pathology clinical features treatment and prevention—and each of these subdivisions is linked with vast comparable sections relating to other diseases The result is that information on for instance the pathology of a tropical disease may be found in a journal of general pathology where its author may have judged that it was most appropriately placed Transmission of many tropical diseases moreover entails the study of intermediate hosts and also of mammalian reservoir hosts their biology and geographical distribution and these studies are often recorded in purely zoological journals In the field of tropical medicine in fact there are large numbers of nonmedical biologists who have added distinction to their subjects and have often brought a scientific precision to the work which has been an example to their medical colleagues It would be invidious to select names but American readers like British readers will have no difficulty in thinking of instances

The study of animal hosts and vectors of tropical parasites has given rise to a large literature of its own but the study of the human hosts and their ecology has not in my opinion received the same attention I have already remarked on the beliefs and traditional customs which influence the people of tropical countries in their acceptance or rejection of Western ideas of disease but the subject goes further The physical changes which take place in the mode of life of these people in the face of industrialization and population pressure lead to severe disease conditions of which perhaps the chief are malnutrition tuberculosis venereal diseases and intestinal infections The point I wish to make is that the answer to these conditions is only partly medical it is largely administrative political and educational and for its study the assistance of the anthropologist is essential This introduces a whole field of literature now only slightly touched but one which may be expected to grow vigorously The physician will remain a key figure in this growth in that he will be the person to see the physical and mental effects of industrialization and political change and will have the duty of drawing the attention of administrators to these effects and of demanding appropriate action

To sum up information on tropical medicine comes from all the usual medical disciplines including psychology and also from a selected but wide field of zoology and from anthropology

This information is contained in medical and scientific journals monographs reports and textbooks

II Medical Journals

A Journals of Wide Circulation

It is quite impracticable in this article to give a useful list of journals published throughout the world in which important papers on tropical medicine may be found a comprehensive list would be so long that it would be useless

In the most recent edition of *World Medical Periodicals* (World Medical Association 1957) the periodicals are listed alphabetically but there is an index in which they are grouped according to subject the journals in each subject being subdivided according to country of origin Under the heading of Tropical Medicine there are 30 current and important journals in English French German Dutch Flemish Italian Spanish Portuguese and Polish languages But on reading through these titles it at once becomes evident that they cover only a small part of what we call tropical medicine The subject of malaria for instance is very largely dealt with in these journals but there are in addition 7 others devoted entirely to it Moreover there is no entry in the index under headings of entomology medical entomology or zoology though some entomological journals are included in the text yet much malaria work is recorded in zoological journals Similarly there are 18 journals on leprosy 34 on parasitology and many (too numerous to be worth counting) on public health infectious diseases general medicine experimental medicine microbiology mycology nutrition and ophthalmology and in almost all of these occasional (and sometimes important) papers relating to tropical medicine may be published

The position seems to be chaotic but the reasons for this great diversity of publications are obvious In the first place as I have already said most of the diseases conventionally regarded as tropical occur also in temperate climates and may be of particular importance to medical men and scientists who do not consider their field to be necessarily tropical For instance some rickettsial diseases are common in the temperate zones and have constituted great public health problems in conditions of extreme cold whereas others like scrub typhus are most in evidence in hot countries plague is enzootic in the United States and in temperate Asia as well as in the Tropics and malaria has a history in Russia and the Netherlands and in the Mississippi Valley as well as in India and Africa

In the second place the object of publication of a particular paper may

be to educate the physicians of a temperate country in the diagnosis and treatment of a disease which may be introduced from abroad For instance after both world wars and after service in Korea service personnel returned to Britain the United States and elsewhere with latent malaria which only too often remained undiagnosed and untreated because unsuspected Papers on malaria were therefore published in the general medical journals of those countries

In the third place some of the discoveries in medicine and parasitology have been sufficiently important to justify early publication in journals of wide circulation and these are usually the journals of general medicine For instance the first records of the discovery of the exoerythrocytic schizonts of malaria parasites of mammals were published in the *British Medical Journal* and similar early announcements of new work are regularly made in *Nature* or *Science*

In the fourth place certain set lectures are by custom published in general medical journals and these lectures sometimes have a tropical bent For instance Bruce s Croonian Lectures to the Royal College of Physicians were published in the *Lancet* in 1915 but they dealt exclusively with African trypanosomiasis

Medical journals are of various kinds Some cater for the publication of original work and these usually also publish occasional papers which are in fact summaries or discussions of work already published (as in the case of Bruce s lectures or the surveys of literature carried regularly in the *American Journal of the Medical Sciences*) or they may carry editorial matter which serves a similar purpose But many of them particularly those published monthly restrict themselves to publication of original papers or addresses without editorial comment

These journals are the main props of medical and scientific research for although much of the material they contain consists of accounts of extension of work already done these are the journals in which vital new discoveries are commonly first made public to which succeeding workers must turn for full grasp of the methods used and the conclusions drawn It is I think important to publish new work in the recognized journals rather than as separate monographs unconnected with any series because the recognized journals are kept in libraries and are easy to refer to and papers in them are part of the main stream of the literature whereas separate publications tend to get lost and therefore are disregarded

Some authors regrettably publish the same work in two or even more journals sometimes dressed up in different words This is justified if it is done for a definite and reasonable purpose (for instance if one version is in a different language or if the original publication will not reach

the desired public) but only if it is stated clearly that the paper has appeared elsewhere Otherwise multiple publication is bad

I have indicated that there are vast numbers of journals in which information on any branch of medicine may be found and because of this there is need for indexing organizations to bring relevant papers to the attention of workers who cannot scrutinize all the journals for themselves

One of these indexing organizations is the United States Public Health Service which issues each month the *Current List of Medical Literature* which under the headings of the principal medical journals of the world (arranged alphabetically) carries the authors and titles of all articles published Each issue has an author index and a subject index This is extremely useful it is accurate and publication is reasonably prompt But it contains only titles without synopses and its value therefore is only that it indicates the content of papers in the briefest manner

The second form of indexing publication is that of the abstracting organization There are many of these and their object may again be to indicate to the reader the papers he should read in full (as in *Chemical Abstracts* where only a few brief notes are given for each paper abstracted) or the object may be to give as much detail as will enable a reader to assess more fully the extent of the work reported in the original and its value or even to give him enough information to enable him to repeat the work This kind of abstract is particularly valuable for readers who cannot consult the original papers either because they are not in sufficient touch with libraries (as in the case of many workers in the Tropics) or because they do not read the original language Abstracting journals which provide only the short abstracts usually attempt to cover the entire literature and they abstract (or quote by title) all available original papers whether good or bad new or old Abstracting journals which provide the fuller synopses of original work are usually much more selective and critical they ignore poor or unduly repetitive work and the abstracts themselves are often critical in the sense that the abstractor may draw attention to flaws in the original or deliberately give an assessment of value The *Tropical Diseases bulletin* performs this function for tropical medicine and has done so for almost fifty years It contains not only abstracts of original papers but also from time to time critical reviews of the literature on selected subjects and regularly summaries of recent abstracts on nine major tropical diseases or groups of diseases It is therefore possible to trace with reasonable ease important work on these subjects back almost to the beginning of the century A similar function

has been performed since 1926 by the *Bulletin of Hygiene* for public health infectious diseases industrial medicine and bacteriology

There are many other abstracting journals From the United Kingdom may be mentioned *Helminthological Abstracts* the *Review of Applied Entomology* the *Review of Medical and Veterinary Mycology* the *Veterinary Bulletin* the *International Abstracts of Biological Sciences* and *Abstracts of World Medicine* From the Netherlands comes *Excerpta Medica* from France the *Bulletin de l Institut Pasteur* from Germany the *Zentralblatt fur Bakteriologie Parasitenkunde Infektionskrankheiten und Hygiene Referate* from the United States *Biological Abstracts* and from Russia *Meditsinskii Referativnii Zhurnal*

All these are valuable and most of the abstracts in them are written by experts in the various fields But it need hardly be said that no abstract is a satisfactory substitute for an original paper and that wherever possible the original should be read Nevertheless the abstracting journals are essential and valuable tools for practicing physicians and research workers alike and they perform a most useful function in enabling administrators and others to keep themselves informed of movements in medicine outside their own immediate specialties

The next stage in the process of recording and organizing information is the publication of reviews of the literature on restricted subjects or monographs In these the author assembles information published over a period of years and usually reviews only those papers which appear to be important relating them to each other in an orderly and systematic way The result is a conspectus of recent work arranged in logical order which embodies a critical assessment of the value of the work reviewed which is most useful for the student *The reviews which will appear in the present publication will be of this kind they form an important and essential bridge between original publications and final textbooks* and they permit mental stocktaking which may be invaluable in clarifying the issues and even in indicating new openings for research

The monograph is an amplification of the critical review its subject matter is more extensive and it aims at presenting a full account of its subject rather than a review of recent work only Two monographs of this kind have recently been published in the Memoir Series of the London School of Hygiene and Tropical Medicine namely *The Natural History of Tsetse Flies* by the late Professor P A Buxton and *Studies on the Exo Erythrocytic Cycle in the Genus Plasmodium* by R S Bray Each of these authors was an authority on his subject and each monograph will remain a fundamental source of authoritative information for succeeding workers

The final stage through which published information passes is that of the general textbook and it is obvious that by the time this is reached much of the work recorded is old though not necessarily invalid on that account Textbooks may be cast in the form of recent advances in a wide range of subjects—for instance recent advances in tropical medicine as a whole—or in the form of comprehensive treatises which set out the elements of their subject fully as in the well known textbooks for students

B Local Medical Journals

Many of the great medical journals are now so well established and so widely read that they attract papers from all over the world because authors realize that in these journals their work has the chance of reaching a universal public But pressure on space is very great in these journals and work which may be regarded as of only local interest to some far off country may have little chance of publication Partly for this reason and partly to encourage the reporting of local work and conditions medical journals have been started in many countries in a rush of enthusiasm These journals at first exist on papers contributed by outside authors—often men of international reputation—who are asked to write specially for them and who do so out of good will The papers they contribute are usually general reviews or reflections on current problems and they rarely contain new facts Later the editor of such a journal finds difficulty in filling his space and there is a danger at this point that the journal may fail and die but if this can be avoided and if the editor can infuse enough life and originality into it to attract local authors to publish in it rather than to attempt publication elsewhere the journal may persist to become a locally interesting and internationally important organ for reflecting local progress I believe that these local journals should be supported and encouraged but the editors should take their work seriously and insist on high standards of work and writing Many such journals have become well established but they need support from local authors

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Two other sources of information which do not quite fit into the categories mentioned above have been instituted by the World Health Organization One of these consists of the published reports of the various Expert Committees of that body and these contain opinions and proposals rather than new work Nevertheless in that they tend to crystallize current views or even to standardize current practice they are most valuable sources of information The other category is less satisfactory from the

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point of view of public information though undoubtedly valuable for its own purposes it consists of the mimeographed *papers circulated to* working parties These are not strictly speaking published in that they are not available to everybody who wishes to have them and they should not therefore be quoted or used as references They are working papers and they may not represent the considered views of the working parties the World Health Organization I understand deprecates quotation from them unless they are printed and published in the usual way But there *is a tendency on the part of* some workers to quote from such documents though this is a practice to be strongly discouraged

The United Nations and Associated World Health Organization are also sources of valuable information through the *publications listed below*

1 United Nations Demographic Yearbook
2 United Nations Statistical Yearbook
3 World Health Organization Annual Epidemiological and Vital Statistics
4 World Health Organization Official Records No 94 First Report on the World Health Situation
5 United Nations Information from Non Self Governing Territories
6 United Nations Food and Agriculture Organization—various publications particularly The State of Food and Agriculture
7 *World Health Organization—annual reports of regional directors*

The Pan American Sanitary Bureau Summary of Four Year Reports on Health Conditions in the Americas are also valuable

Annual reports of government departments research organizations local authority departments and other bodies of course all may constitute valuable sources of information even if the facts they publish have already been the subject of individual articles From the point of view of epidemiology and the trends of disease for instance the annual statistical reports of the Ministries of Health or the Medical Departments are invaluable They are carefully prepared and the information they contain can be compared *with similar information in reports of former years* so that estimates of progress can be made Conditions in one country can also be compared with conditions in others so long as it is understood that the medical services of most of these countries are still far from complete and that the amount of disease found depends on the numbers of medical personnel looking for it

Annual reports of research institutions are important They often summarize the work done *even though the full reports of that work appear* independently in medical journals For many years the Pasteur Institutes have issued reports of this kind from Paris North Africa other

French African Territories Indochina Martinique and French Guiana and also from India (Coonoor) Persia and certain other countries Many distinguished French scientists have worked in these institutes and the subjects they have covered range far beyond the rabies which has always been a particular interest Similarly important records of routine work and research have been issued periodically from such centers as the Gorgas Memorial Laboratory Panama the Instituto Butantan Sao Paulo Brazil the Instituto Oswaldo Cruz Rio de Janeiro the South African Institute for Medical Research the Onderstepoort Institute of Veterinary Research South Africa the King Institute Guindy India the Institute for Medical Research Kuala Lumpur Malaya (which has also issued a remarkable series of special reports on various investigations) Since World War II the British African countries have reorganized and developed their research institutions extensively These institutions issue annual reports They include the East African Medical Survey the East African Virus Research Institute (formerly the Yellow Fever Research Institute) the East African Institute for Malaria and the Vector Borne Diseases the East African Trypanosomiasis Research Organization the Filariasis Research Unit These East African units are under the control of the East Africa High Commission and are closely associated with the Colonial Medical Research Committee whose headquarters are in London In West Africa there are the *West African Virus Research Institute (formerly the Yellow Fever Institute)* and the West African Institute for Trypanosomiasis Research In addition there was the malaria research department of the health services of Nigeria before that country was divided into the three regions which are now autonomous In Gambia there is also a research unit of the Medical Research Council which is making a long term nutritional and medical survey

All these units contribute to our knowledge of tropical conditions by their annual reports which are careful and informative documents of great value in which the most recent developments are briefly described It should be understood that many of these units are the successors of other organizations which were set up by the governments of the various countries—Uganda Kenya Tanganyika Nigeria Ghana Sierra Leone Gambia—many years ago The movement toward unification of research effort has recently been very marked

For Belgian dependencies the Institut pour la Recherche Scientifique *en Afrique Centrale (IRSAC) was founded in 1947 and issues annual* reports it covers all branches of science The special organization known as FOREAMI (Fonds Reine Elisabeth pour l'Assistance Medicale aux Indigenes) also issues its own informative reports and outside govern

ment the Union Miniere du Haut Katanga gives annually an account of its medical services which is of particular value since the personnel of this mining company forms in the main a stable labor force in which there is excellent opportunity for observing not only the employees but also their families The reports of the Union Miniere are most valuable

Similar reports from mining organizations are issued from Johannes burg (Central Mining Rand Mines Group Health Department) and by the Silicosis Medical Bureau of Northern Rhodesia

For the student who wishes to go back into the history of his subject or to follow the work of one particular author the famous American compilation known colloquially as Stiles and Hassall (*Index Catalogue of Medical and Veterinary Zoology*) is most satisfactory It deals only with medical and veterinary parasitology but the succeeding parts issued at intervals list by title and reference the works of authors all over the world in those chosen subjects and the earlier volumes took this history back quite a long way Bibliographies of this kind including the even more famous *Quarterly Cumulative Index Medicus* (now super seded by the *Current List of Medical Literature*) and the *Index Catalogue of the Library of the Surgeon General s Office U S Army* remain of the greatest value for those for wish to inform themselves of early work on their subjects

Bibliography would appear to be dull work though it has attracted some acute minds but it has a permanent value So many bibliographies have been compiled either on the basis of individual authors or individual subjects that it would be hopeless to attempt to list them but mention may be made of the *Zoological Record* which has an interest for tropical medicine the *Bibliography of Trypanosomiasis* by C A Thimm published in London in 1909 by the Sleeping Sickness Bureau and the *Bibliography of Schistosomiasis* by M Khalil published in Cairo in 1931 and followed in 1950 by the *Bibliographie des Schistosomes et des Schistosomiases (Bilharzioses) Humaines et Animales* by A Bouillon published in Brussels These and similar bibliographies are careful and in the main exact lists of references compiled with painstaking labor and providing guides to the student and historian

There have been numerous historical studies in tropical medicine but the greatest and most comprehensive was published in 1939 by my pred ecessor—*A History of Tropical Medicine* by H Harold Scott—and I regard this as indispensable reading for anybody who wishes to understand the subject

IV Presentation

A Form

Modern medical and scientific investigation moves at such a pace and is pursued along similar lines in so many parts of the world at once that scientists are more eager than ever before to keep up to date with the latest developments In the reverse direction therefore they are equally eager to make known their own findings as quickly as possible and the proper and orthodox method of doing so is by publication in scientific journals The flow of information constantly tends to outstrip the capacity of existing journals so that at the present time some of the most reputable of them have waiting lists of up to a year for the publication of material submitted to them This is a most unsatisfactory situation and some journals therefore have developed a system whereby they publish letters to the editor which contain preliminary announcements of work done These are often followed by full accounts of the work published in the same or in different journals several months later

Journals which publish preliminary material in this way include *Nature* *Science* the *Transactions of the Royal Society of Tropical Medicine and Hygiene* the *American Review of Tuberculosis* and many others but they too are now beginning to develop waiting lists as a result of heavy pressure of this type of communication

A second type of journal caters for short communications that sometimes may be sufficiently important to warrant rapid publication but may also carry brief papers on less urgent material Instances are the *Proceedings of the Society for Experimental Biology and Medicine* the *Bulletin de la Société de Pathologie Exotique* and the *Comptes Rendus de la Société de Biologie* The papers published in these are almost invariably short they deal usualy with relatively small investigations and quite often they are later followed by sequels dealing with further investigations on the same subject

The justification for these letters to the editor and these short restricted papers is that they make known important work quickly But it is unfortunately true that there is a very widespread tendency to publish hort papers in series on work which could much more effectively have waited until it could be reported in full in a single paper A second justification is to establish priority which is permissible if the occasion is serious enough *But it should not be overdone My own feeling on this* question of short communications is that they should if possible be avoided unless they are of outstanding importance and especially if they form part of a larger body of work which will eventually need to be brought together

The *third type of paper forms the* backbone of scientific research it is the full deliberate record of a piece of work which has reached a stage of completion at which publication is seen to be appropriate This is not to say that it is the end of the story and that nothing more remains to be done *but it is to say* that the work has at least reached a major pause and that further work would demand considerable thought and effort Most of the great advances in medicine have been recorded in substantial writings of this kind and in fact this kind of paper is in the mind of the *author* who makes *his short preliminary announcement in a letter* to the editor to establish priority or to give his contemporaries early information on his work He follows up his first note with a full detailed paper

After the full detailed paper which *may be linked in subject matter* with later work by the same author or with similar work by others comes the conference paper in which the same ground is covered A conference is not usually the place at which new discoveries are first an*nounced though this may be done if time and place permit as when* R B Heisch at the conference in Lisbon in 1958 (VIth International Congresses of Tropical Medicine and Malaria) announced the successful infection for the first time of man with trypanosomes (*Trypanosoma rhodesiense*) *isolated directly from a mammal* (bushbuck) shot in the wild state in Kenya But an announcement of this kind could not expect to receive publication until perhaps one or two years after the conference has ended when the proceedings are eventually issued and *it is necessary* in such an event to ensure valid recording by publishing the work independently in a recognized journal This was done by Heisch and his colleagues in the *British Medical Journal* (1958 Nov 15 p 1203)

Conference papers nevertheless are very *useful in* that they are published along with other contributions made at the same time on the same subject and they form convenient conspectuses of current opinion usefully brought together and easily found They do not when published pretend *to be completely* up to date *and of course they* tend to be fragmentary in that they rarely cover the whole of the subjects with which they deal

Reviews of the literature have been referred to above In one form the *author sums up the literature periodically* so that any worker *wishing to trace a subject has at his disposal a series of reviews* organized into logical sections which he can follow with little trouble These reviews include references to the original papers so that the reader is easily made aware of the sources Reviews *of this kind have been published in the Tropical Diseases Bulletin* regularly each year since 1939 on the nine main groups of tropical diseases and on venereal diseases (*Perspectives in Venereol*

ogy) in the *Bulletin of Hygiene* since 1948 The reviews in the *Tropical Diseases Bulletin* are regularly translated into French and published in *Médecine Tropicale*

Reviews of this kind are critical in the sense that inferior or unimportant work is not included and they do occasionally carry some critical comment but their main function is to act as informed guides to the literature

The other form of critical review such as appears in *Bacteriological Reviews* and many other publications has been discussed above under the heading of Medical Journals

B STYLE

The technique of presentation does not I think receive as much attention as it needs and though in some respects we have improved our standards in others we have retrogressed I have recently been reading *An Essay Concerning the Cause of the Endemial Colic of Devonshire* by George Baker first published in 1767 This short classic of English medical investigation has recently been issued in facsimile form by the American Public Health Association through the initiative of Dr Huntington Williams of Baltimore It illustrates my point We have now I think improved our layout in that most of the papers we publish are divided into logical sections well distinguished by headings and sub headings and in this way we make it easy for a reader to find and refer back to the sections on methods materials results discussion etc But we have lost clarity in our use of language partly because we tend to think that the technique of writing is unimportant partly because we do not pay enough attention to the needs of the readers and partly because we have gone too far in compression Brevity in general is a virtue but like other virtues it can be made oppressive Baker s language is leisurely though not ponderous and it is interspersed by extensive quotations in Latin but there is never any need to reread a passage because of obscurity of language This is not true of some of our modern writing and the reason is not always the complexity of the subject It is true of course that medical research is now very complicated and that the facts and their implications are often hard to grasp but difficult concepts have always existed and have been expressed and some of our modern writers are as clear as their predecessors showing that clarity is possible I believe that clarity in writing is not so much an inborn gift as the result of care and imagination on the part of the writer

In scientific writing clarity and interest are the two essential qualities The interest depends very largely on the subject matter and the intelligence of the author in his approach to it but interest can be lost if the

presentation is poor and clear language is the first essential of good presentation

Many papers and books have been written on the writing of scientific papers and the excellent guidance they give is largely concerned with details of technique and grammar The present paper is not the place for such advice but broad principles may be stated again—they are far too often ignored by authors Editors of journals—hard working and self effacing persons—rarely receive the credit they deserve for adding point and sparkle to dull papers but authors should not rely too much on their editors An editor is rarely expert in all the subjects of the papers he edits and may in seeking good presentation change the emphasis or even the meaning of the original The author will see the paper in proof but I have known that kind of editorial change of meaning to escape their notice Moreover it is well known that an author clarifies his own mind in the process of writing clearly and he should cultivate clear writing for this as well as for other reasons

Scientific writing should be crisp and vigorous which implies among other things that it should not be weighted down with cliches and jargon Writing is not the same as speech for in speech the emphasis can be varied by tone of voice by pauses and even by repetition which are impossible or inappropriate in print There is therefore a danger in dictating material for publication and my own feeling and experience is that the scientist composes best when he writes deliberately in longhand correcting as he goes along Writing of that kind is laborious but is worth the effort if what is written is meant to have more than a temporary value

Jargon can be a great disadvantage It is commonly used in conversation in laboratories and hospitals and there it is permissible as conversational shorthand but it should be most carefully controlled in writing In the first place foreign readers may be completely defeated by it—and here I include the now excessive use of initial letters especially if they are not explained at least once in the text when first used In the second place readers not working in the same field but who wish to keep themselves informed are likely to be confused I have in mind medical administrators and teachers who need to know the outlines of subjects impinging on their own In the third place today s jargon may be forgotten tomorrow Writing should always be persuasive and should induce the reader to go on but it cannot do this if the jargon is obscure

Writing gains in persuasiveness if it has a personal element to warm it and I have a feeling that we have become too impersonal and dry in our approach to publication I do not mean that the style should be flamboyant but the personal touch perhaps in recounting some illustra

tive incident or in giving an opinion in vigorous terms embellishes a paper if it is not overdone I believe that a statement of opinion on a debatable point and a personal discussion of the implications of a piece of work are essential features of good writing They are usually appropriate

A scientific paper therefore should be well thought out in advance and should be well organized and divided into a logical sequence of sections There are innumerable variations of pattern but in general it will be possible to state the problem describe the materials and methods employed and the results obtained and to discuss their implications And all papers should include an informative but brief summary The work should be linked with other relevant work briefly if possible but in any event clearly so that to the reasonably informed reader it can stand by itself

Much more could be written on the details of presentation but this is not the place for it Most of the advice is common sense and is not needed by authors who take the trouble to put themselves in their readers place and who write as if they intend their writing to have permanent value to be read and reread as significant medical literature I have read many papers conceived and written on the highest level they are not only works of scientific value they are stimulating works of art

V Summary

1 Tropical medicine is discussed as a subject which embraces most human diseases though with emphasis on those particularly prevalent in hot countries and which requires an extensive knowledge of zoology and other disciplines

2 Publications containing information relating to tropical medicine are discussed they include journals specializing in diseases of hot climates but most other medical journals carry occasional papers important to the subject They also include reports from various bodies Original papers conference papers abstracts reviews and textbooks are briefly discussed

3 The importance of presentation is discussed with special reference to style in writing

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